TRAINING UPDATE

Lab Location:	GEC, SGAH & WAH	**Date Distributed:**	9/26/2014
Department:	Core	**Due Date:**	10/31/2014
		Implementation:	**11/1/2014**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Coulter LH750 Operation for Complete Blood Count and Reticulocyte Automated Tests

GEC.H217 v2, SGAH.H01, WAH.H01 v5

Description of change(s):

Sections10.3, 11.2, Addenda 1:
Replaced 103/µL units with x10(3)/mcL

Note: This change was made in LIS on 9/24/14 in support of a Cerner project

The revised SOP will be implemented on November 1, 2014

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

Approved draft for training

Technical SOP

Title	**Coulter LH750 Operation for Complete Blood Count and Reticulocyte Automated Tests**
Prepared by	Robert SanLuis, Leslie Barrett Date: 9/28/2009
Owner	Robert SanLuis Date: 9/21/2012

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name	**Signature**	**Date**

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Quest Diagnostics
Site: GEC, SGAH & WAH
Title: **Coulter LH750 Operation for Complete Blood Count and Reticulocyte Automated Tests**

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Hemogram *(WBC, RBC, HGB, HCT, MCV, MCH, MCHC ,RDW PLT, MPV)*	Coulter Automated Hematology Analyzer, LH750	CBCND
Hemogram & diff (*WBC,RBC,HGB,HCT,MCV,MCH,MCHC,RDW, PLT, MPV, differential)*		CBC
Differential count only		DIFF
Platelet Count		PLTC
Reticulocyte Count		RETA

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Abbreviation	Term	Abbreviation	Term
WBC	White Blood Cell	MCHC	Mean Corpuscular Hemoglobin Concentration
RBC	Red Blood Cell		
HGB	Hemoglobin	RDW	Red Cell distribution Width
HCT	Hematocrit	DIFF	Differential Count
MCV	Mean Cell Volume	PLT	Platelet
MCH	Mean Corpuscular Hemoglobin	MPV	Mean Platelet Volume
RETIC	Reticulocyte Count		

Department
Hematology

2. ANALYTICAL PRINCIPLE

CBC
The Coulter principle employs electronic counting and sizing of particles using the LH 750 Series Hematology analyzers. WBC Differential analysis and classification are based on simultaneous measuring of cell volume, high frequency conductivity and laser light Scatter. Hemoglobin, released by hemolysis, is converted to a stable cyanide containing pigment and measured by photometric absorbance.

Reticulocyte
Red blood cell *(RBC)* RNA is stained with the vital stain new methylene blue. The dye precipitates the RNA found in reticulated RBC. Hemoglobin is removed from the RBC leaving the precipitated dye-RNA complex by adding a sulfuric acid solution. Reticulocyte percent and number are measured by analysis of the total RBC population for volume, conductivity and light scatter.

2.1 Determination of Parameters

Type of Measurement	Parameter	Source of Data
Direct	RBC *(Red Blood Cell)*	Coulter principle
	WBC *(White Blood Cell)*	Coulter principle
	HGB *(Hemoglobin)*	Photometric absorbance
	MCV *(Mean Cell Volume)*	Coulter principle
	PLT (Platelet)	Coulter principle
	Automated Differential, five-part	Light scatter, volume & conductivity (VCS technology)
	RET% (Reticulocyte)	VCS Technology
Derived from Histograms	RDW *(RBC Distribution Width)*	RBC Histogram
	MPV *(Mean Platelet Volume)*	PLT Histogram
	NRBC%	WBC Histogram and VCS technology
Calculated	HCT *(Hematocrit)*	HCT = $\frac{\text{RBC x MCV}}{10}$
	MCH *(Mean Corpuscular Hemoglobin)*	MCH = $\frac{\text{HGB x 10}}{\text{RBC}}$
	MCHC *(Mean Hemoglobin Concentration)*	MCHC = $\frac{\text{HGB x 100}}{\text{HCT}}$
	DIFF # parameters	DIFF as % x WBC (i.e. 0.77 x 5800)
	Absolute Neut	(Neut% + Band% + Meta% + Myelo% + Promyelo%) X WBC
	Absolute Lymph	(Lymph% + Reactive Lymph%) X WBC
	Absolute Monocytes	Mono% X WBC
	Absolute Eosinophils	Eos% X WBC
	Absolute Basophils	Baso% X WBC

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3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	Not applicable
Specimen Collection and/or Timing	None defined
Special Collection Procedures	None defined

3.2 Specimen Type & Handling

Criteria			
Type -Preferred -Other Acceptable	K_3EDTA or K_2EDTA Whole Blood Sodium Citrate – for platelet counts only		
Collection Container	Lavender Top Tube Tri-Potassium or Di-Potassium EDTA Anticoagulant		
Volume	**Tube**	**Minimum**	**Optimum**
	K_3EDTA or K_2EDTA (non-pediatric)	1.0mL	Full tube
	Pediatric K_3EDTA or K_2EDTA tube	0.5mL	Full tube
	Microtainer tube	0.5mL	n/a
Transport Container and Temperature	Same as above. Transport at room temperature or refrigerated.		

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Stability & Storage Requirements	Room Temperature (18-25ºC): 48 hours Refrigerated: After analysis, specimens are stored for a minimum of 2 days at 2-8°C. Frozen (-20ºC and below): Not Acceptable			
Timing Considerations	N/A			
Specimen Quality Table	**Condition**	**Slight**	**Moderate**	**Marked**
	Icterus	OK	OK	Orange-Brown = *see section 13.8*
	Hemolysis	Slight pink OK	Pink OK	Cherry Red Unacceptable
	Lipemia	OK	OK	Milky = *see section 13.8*
Other Interfering Specimens Factors	**CBC** Indicated by CBC results *(see Addendum 2)* Fibrin, bacterial contamination, platelet clumps, abnormal proteins, cold agglutinins, extreme temperature conditions, resistant hemoglobin, abnormal chemistries and specimens older than 48 hours. **RETIC** Extreme temperatures, other erythrocyte inclusions that stain by new methylene blue dye, some hemoglobinopathies *(SS, SC),* and specimens older than 72 hours.			
Actions to Take for Rejected Specimens Message Codes & Notes	**Condition**	**Code**	**Comment**	
	QNS *(Less than the minimum volume in Section 3.2)*	QNS	Quantity not sufficient to perform test. **Notify caregiver**. *(Document in the LIS)*	
	Clotted	CLT	Specimen is clotted, unable to perform test. **Notify caregiver.** *(Document in the LIS)*	
	Spurious results that will not duplicate	INT *or* UNSAT	Possible interfering substance. *or* Unsatisfactory specimen. **Notify caregiver.** *(Document in the LIS)*	
	Gross hemolysis	HMT	Markedly hemolyzed. **Notify caregiver.** *(Document in the LIS)*	

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents	Stability *(Opened)*	Supplier & Catalog Number	Size
Lyse S III diff	60 days	Beckman Coulter – Cat # 8546796	5 Liter
Coulter Clenz	90 days	Beckman Coulter – Cat # 8546931	10 Liter
LH 700 Series Diluent	60 days	Beckman Coulter - PN # 8547194	20 Liter
LH 700 Series PAK	60 days	Beckman Coulter - PN # 8547195	N/A
LH 700 Series Retic PAK	60 days	Beckman Coulter - PN # 8547196	N/A

4.2 Reagent Preparation and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.
Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Reagent	Lyse S III diff, LH 700 Series Diluent, LH 700 Series Retic PAK
Storage	2-30ºC
Stability	Stable (when unopened) until expiration date on label.
Preparation	All reagents are received ready for use.

Reagent	Coulter Clenz, LH 700 Series PAK
Storage	2-25ºC
Stability	Stable (when unopened) until expiration date on label.
Preparation	All reagents are received ready for use.

4.3 Diluents and lysing agents should be checked to be sure that no interferences are present. Performing a background count is an effective way to detect interference. Daily start up process insures that all diluent, lyse and reagents on board have been background checked. If reagents are changed after initial start up, another start up is required to comply. Each time the diluent is changed a background check is performed to insure no bubbles or contamination are introduced that could compromise patient testing. Document all reagent changes/background checks as specified in addendum 7.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier & Catalog Number
Coulter® S-Cal® Calibrator Kit	Beckman Coulter, PN 7508116-A

Caution: Calibrator contains sodium azide (<0.1 %).
Contains potentially biohazardous materials.
Use with good laboratory practices to avoid skin/eye contact or ingestion.
Consult MSDS for a complete list of hazards

5.2 Calibrator Preparation and Storage

NOTE: Date and initial all calibrators upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech (6) any special storage instructions; check for visible signs of degradation.

Calibrator	Coulter® S-Cal® Calibrator Kit
Preparation	Bring to room temperature prior to testing. Use within one hour.
Storage/Stability	Store refrigerated (2-8°C). Use within expiration date from manufacturer. For further details refer to the package insert. Check for visible signs of degradation prior to use, i.e. color change or clotting.

5.3 Calibration Procedure

Criteria	Special Notations
Frequency	At least every 6 months, and when indicated by the following: • New set of apertures is installed. • New blood sampling valve is installed. • New electronics are installed. • When multiple levels of commercial controls are consistently out or biased for one or more parameters. NOTE: Calibration is performed in the closed mode Calibration must be verified for both sampling modes, opened and closed *(cap-piercer)*. . When any parameter is adjusted, the change must be made or verified for both sampling modes.

Criteria	Special Notations	
Calibration Preparation	Before Calibration: • Has instrument had PM in the last 6 months (*Consult Supervisor)* • Verify all routine maintenance is up-to-date. • Clean the Baths. • Perform shutdown. • Ensure you have sufficient supply of reagents to complete the procedure. • Perform Startup. • Perform Reproducibility: ***1**-If the CV% for any parameter is greater than those listed; you might have an instrument problem. Call your Coulter Representative.* ***2**-Review each parameter for trending (a gradual and consistent increase or decrease in values). If you think a trend exists, you might have an instrument problem; call your Coulter Representative.* • Perform Carryover Check: *(Validate carryover (%) for each parameter against manufacturer acceptability guidelines; if exceeded, call your Coulter Representative).* • If all of the above are determined to be acceptable, then proceed with S-Calibration. Otherwise, correct the deficiency and repeat the reproducibility & carryover procedures. • Follow the S-Cal preparation, handling, and procedural instructions.	
Tolerance Limits	**IF …**	**Then …**
	If results fall within the specifications, if calibration status is displayed as acceptable and Quality Control (QC) values are within acceptable limits.	Proceed with analysis.
	If results fall outside of specifications and the calibration status is displayed as failed or the QC values are outside acceptable limits.	Troubleshoot the assay and/or instrument and repeat the calibration.
	If repeat calibration fails,	Contact Beckman Coulter for technical support.
Procedure	Follow instructions in the current S-Cal package and/or refer to the LH750 Calibration Screen Help Procedure.	

5.4 Documentation

All Calibration and/or Calibration Verification processes (with commercial material) are documented. Calibration and/or Calibration Verification processes are signed and dated by performing staff. Calibration and/or Calibration Verification documents are reviewed, dated, and signed by supervisory staff. Calibration and/or Calibration Verification documents are QC documents and maintained according to guidelines published in the Quest Diagnostics *Records Management Program Reference Guide.*

6. QUALITY CONTROL

6.1 Controls Used

Caution: Controls contain sodium azide (<0.1 %).
Potential biohazardous materials.
Use with good laboratory practices to avoid skin/eye contact or ingestion.
Consult MSDS for a complete list of hazards.

Control	Supplier & Product Number
5C Abnormal I 5C Normal 5C Abnormal II	Beckman Coulter # 7547116 4 x 3.3 mL each level
RETIC – C (Level I, II, III)	Beckman Coulter # 7547125 3 x 3.3 mL each level
Latron 1 *(primer)*	Beckman Coulter # 7546915 5 x 16 mL each
Latron 2 *(control)*	Beckman Coulter # 7546914 5 x 16 mL each

6.2 Control Preparation and Storage

NOTE: No control preparation is necessary. Follow instructions in the current control package insert for control handling. Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation. Follow the QC Program when checking new lots or shipments of QC material prior to use.

Control	Storage & Stability
5C Abnormal I **5C Normal** **5C Abnormal II**	• Store refrigerated at 2-8°C. • Bring to room temperature prior to testing. • Observe expiration date. • Open vial stability: 13 days or 13 uses.
Latron 1 *(primer)*	• Store at 2-30°C. • Bring to room temperature prior to testing. • Observe expiration date. • Open vial stability: 30 days
Latron 2 *(control)*	• Store at 2-30°C. • Bring to room temperature prior to testing. • Open vial stability : 30 days
RETIC-C (Levels I, II, III)	• Store refrigerated at 2-8°C. • Bring to room temperature prior to testing. • Observe expiration date. • Open vial stability: 15 days

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6.3 Frequency

A. All three commercial control levels of 5C and Retic must be tested each shift. Data from this control run is part of the Coulter eIQAP program.

B. Latron Control will be tested as part of start-up procedure only. Refer to Coulter LH750 help screen for Latron Control for procedure.

C. **Multi-mode sampling**
- Each day both closed-mode and open-mode sampling using the 5C controls must be performed, as per CLIA and CAP requirements.
- Typically, most testing is done in closed mode.
- Commercial Controls must be tested in open and closed mode on each shift.

D. See Addendum 4 for the Daily Quality Control Schedule for the Beckman Coulter LH750. *(Note: Due to the QC schedule the 5C on the LH750 gets sampled 11-12 times at the most before it is depleted.)*

6.4 Tolerance Limits

The laboratory's QC program is set up with mean values provided in the package insert for the respective lot# of QC that have been verified per laboratory procedure. For tracking QC in the LH database, Standard Deviations *(SDs)* used for acceptable limits must not exceed the Max SD or the SDc *(determined from the Coulter QC Range),* whichever is greater.

QC Level	Parameter	Max. Total Allowable Error	Max CV, %	Max SD	SDc = Coulter Range / 3
Abnormal I	WBC	+/- 15%	3.0	0.60	0.37
	RBC	+/- 6%	1.2	0.05	0.04
	Hemoglobin	+/- 7%	1.4	0.18	0.13
	Hematocrit	+/- 6%	1.4	0.52	0.63
	MCV	+/- 6%	1.2	1.0	1.0
	Platelet Count	+/- 25%	5.0	17	13
	Neutrophils (%)	+/- 3SD	1.5	1.0	1.67
	Lymphocyte (%)	+/- 3SD	3.0	0.43	1.67
	Monocytes (%)	+/- 3 SD	4.0	0.6	1.0

QC Level	Parameter	Max. Total Allowable Error	Max CV, %	Max SD	SDc = Coulter Range / 3
Normal	WBC	+/- 15%	3.0	0.27	0.27
	RBC	+/- 6%	1.2	0.06	0.06
	Hemoglobin	+/- 7%	1.4	0.23	0.20
	Hematocrit	+/- 6%	1.4	0.67	0.90
	MCV	+/- 6%	1.2	1.0	1.0

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	Platelet Count	+/- 25%	5.0	8.7	8.5
	Neutrophils (%)	+/- 3SD	1.5	0.8	1.67
	Lymphocyte (%)	+/- 3SD	3.0	0.79	1.67
	Monocytes (%)	+/- 3 SD	5.0	0.5	1.0

QC Level	Parameter	Max. Total Allowable Error	Max CV, %	Max SD	SDc = Coulter Range / 3
Abnormal II	WBC	+/- 15%	3.0	0.10	0.13
	RBC	+/- 6%	1.2	0.022	0.027
	Hemoglobin	+/- 7%	1.4	0.07	0.10
	Hematocrit	+/- 6%	1.4	0.21	0.50
	MCV	+/- 6%	1.2	1.0	1.0
	Platelet Count	+/- 25%	5.0	3.0	5.0
	Neutrophils (%)	+/- 3SD	2.4	1.0	1.67
	Lymphocyte (%)	+/- 3SD	2.2	1.0	2.0
	Monocytes (%)	+/- 3 SD	8.0	0.7	1.0

QC Level	Parameter	Max. Total Allowable Error	Max CV %	Max SD	SDc = Coulter Range / 3
Level I	Retic	+/- 3SD	15.4	0.17	0.2
Level II	Retic	+/- 3SD	3.2	0.1	0.4
Level III	Retic	+/- 3SD	4.2	0.4	0.8

Maximum total allowable error is based on CLIA 88 criteria, which also are the CAP evaluation criteria.

Max CV is established by QC BPT to be consistent with recommended QC rules (see part c, below) in order to detect changes in the assay that would cause an error that exceeded the maximum allowable total error.

Max SD is determined by multiplying the maximum CV * assay value. The assay value changes slightly for each new lot, however, it is expected that the precision will remain constant for each new lot of material.

SDc = Coulter Range / 3. This is the value of the SD that would match Coulter Range if we use 3 SD QC limits. In some cases, this SD is very similar to the Max SD, while in other cases, these values differ.

RUN REJECT CRITERIA: The QC procedure for this assay will employ **the 1-3S Westgard rule.** The 3SD limit will be identical to the Coulter QC limit. Runs where this QC rule is violated will be rejected. QC repeated and lookback performed and documented for each out of range parameter.

Each time one control exceeds the criteria for rejection, the run is out of control *(failed)*, and patient results must not be reported. The steps on the QC flow chart must be followed to resolve the problem.

Corrective Action

- Rejected runs must be effectively addressed by corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC protocol. Supervisor may override rejection of partial or complete runs only with detailed documentation that follows criteria that is approved by the Medical Director.
- Corrective action documentation must include the following: QC rule(s) violated, the root cause of the problem, steps taken to correct the problem, how patient samples were handled, and the date and initials of the person recording the information.

Review of QC

- Upon weekly and monthly review of QC, if the QC is showing a shift or a drift investigate the cause for the imprecision and document corrective actions. Monthly QC files are printed, compiled in a log and reviewed by the department supervisor/manager or designee.
- All daily shift QC must be submitted with 5 days of outdating to Coulter's eIQAP program for interlaboratory comparison.

6.5 Review Patient Data

Review patient results for unusual patterns, trends or distributions, looking for an unusually high percentage of abnormal results.

6.6 Documentation

- QC results for each control level and each test mode for an instrument are recorded and stored in the instrument.
- QC records are printed monthly and maintained and available for a minimum of two *(2)* years.
- Patient results are reviewed and released to the patient file via the LIS system.

6.7 Quality Assurance Program

- Refer to the QA / QC policy for other quality assurance activities applicable to this procedure.
- Training must be successfully completed and documented prior to performing this test.
- The laboratory participates in CAP proficiency testing.

6.8 Other QA Tools – XB Moving Averages

XB moving averages should be utilized with caution for specific patient populations as XB results can be skewed.

IF …	THEN …
XB shows a characteristic pattern of an upward or downward drift	• Check patient population to eliminate the possibility of an increased number of patients with a specific disease state. If this is found, then continue to run instrument. • Check patient population, if an increased number of patients with a specific disease state is not found, check commercial material for similar trends/shifts.
If commercial control material is in control	The instrument can continue to be operated.
If the commercial control material shows a similar trend/shift	Troubleshoot the instrument and calibrate if necessary.

Hints For XB Troubleshooting			
When Measurement	Then …		
	MCV	MCH	MCHC
HGB Decreased	No change	Decreased	Decreased
HGB Increased	No change	Increased	Increased
RBC Decreased	Increased	Increased	No change
RBC Increased	Decreased	Decreased	No change
HCT Decreased	Decreased	No change	Increased
HCT Increased	Increased	No change	Decreased

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Brand	Instrument Model	Distributor
Beckman Coulter	LH750	Beckman-Coulter, Inc. Technical Support 1-800-526-7694

7.2 Equipment

Item	Supplier and Catalog Number
Microscope	None specified
Slide Stainer	None specified
Refrigerator, 2-8°C	None specified
Printer	None specified

7.3 Supplies

Other Items	Supplier and Catalog Number
Biohazard wipes	None specified
Immersion Oil	None specified
Applicator sticks	None specified
Glass Slides	None specified
Lens Paper	None specified
Optical lens cleaner	None specified

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8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as Related Documents.

8.1 Preventive Maintenance

Daily and weekly maintenance will be performed and documented on the maintenance log by assigned personnel. Please refer to the LH750 Help Screen.

8.2 Start-up/Shut down Procedure

Refer to the LH750 Help Screen.

8.3 Loading Cassettes

Prior to loading cassettes, mix specimens on a mechanical rocker for 5-10 minutes

Step	Action	
1.	**IF**: Specimen received in standard tube containing optimum amount.	**THEN**: Load cassettes making sure all bar code labels are positioned appropriately.
2.	**IF**: Specimen received in Microtainer tube or contains minimum amount of blood.	**THEN**: Run the specimen in the open mode.
3.	Place the cassettes on the loading bay. The instrument starts automatically when the cassette is placed on the loading bay**.**	
4.	When load is completed, remove cassettes from instrument.	

8.4 Review of Patient Result

Step	Action
1.	Using function OEM in the LIS system, review each patient result before it is released.
2.	Check for delta checks and critical values.
3.	Call and document all critical values

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4.	Release all values that do not need to be repeated for delta values, critical values, or are not flagged on the LH print out for review.
5.	Pull all specimens that need rerun or slide scan as indicated in Addendum 2.
6.	Store normal specimens.
7.	Rerun tests (those needing repeat analysis as indicated in Addendum 2
8.	For those specimens that are flagged for scan smear or perform manual diff, release the hemogram and "Hold" the diff. Refer to Addendum 3
9.	Make slide for scan smear. Refer to Addendum 8.

8.5 Supervisor (or designee)/Pathologist slide review

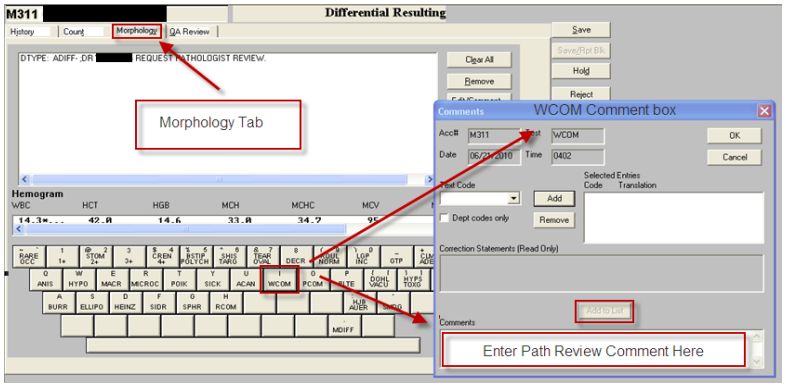
Abnormality	Supv.	Path.
Prolymphs > 5%	X	
Reactive and/or atypical lymphocytes >20%	X	
Bands > 25%	X	
Meta/Myelos/Promyelo >10%	X	
Any blast cell	X	X
Any unidentifiable cell	X	X
Any parasite or micro organism (reviewed by microbiology also)	X	
Lymphocyte > 75% in patients < 17 years of age	X	
Lymphocyte > 70% in patients > 17 years of age	X	X

NOTE: The above guidelines are for new and recurring patients performed initially and over each subsequent hospital encounter (ED visit, OP visit or admission).

8.6 Handling and Resulting Pathologist Reviewed Slides

A. Technician/Technologist will submit slides for pathologist review as follows:
   1. Ensure slide is of acceptable quality for pathology review; appropriate smear, adequate staining, and properly labeled.
   2. Cover-slip the slide
   3. Complete Pathologist Slide Review Request form
   4. Attach analyzer print-out (Scatter Plot)
   5. Print patient cumulative report (LIS procedure SGAH.LIS22 or WAH.LIS22)

B. Technician/Technologist will enter Pathologist comments in LIS as follows:
   1. The pathologist will write comments on the Pathology Review Request form.
   2. The technician/technologist will enter the pathologist's interpretation in the LIS under Differential Result Entry – Morphology Tab - WCOM *(See Example Below)*



3. In the comment box enter, **"Differential reviewed by Dr. (name of pathologist)"** along with pertinent comments as indicated by the reviewing pathologist. **Note:** *All comments must be immediately preceded by a* ***semicolon***.
4. Proof read the comment for grammatical and spelling errors then select "Add to List" the button directly above the comment will highlight once text is entered. **Note:** The comment may be typed into a word document, checked for grammatical and spelling errors, then copied from the word document and pasted into the comment field.
5. Review the comment under the QA Review Tab prior to saving the result.

9. CALCULATIONS

MCV, MCH, MCHC and absolute differential results are released from the LH analyzer.

The absolute differential results are released from either the analyzer or the LIS, depending upon the differential type:

- Automated differentials have the absolute values calculated by the LH750.
- Manual differentials have the absolute values calculated by the LIS.

There are instances when results are above assay range or interfering substances require manual correction of assay parameters. These calculations are verified at least annually as well as whenever a change is made to the LIS that could impact a calculation. See Addendum 5 for calculation formulas.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

Any result rounding is performed at the interface level.

10.3 Units of Measure

Parameter	Units
WBC	x10(3)/mcL or K/µL
RBC	10^6 /µL or M/µL
HGB	g/dL
HCT	%
MCV	fL
MCH	pg
MCHC	g/dL
PLT	x10(3)/mcL or K/µL
MPV	fL
RDW	%
Differential Absolute Values	Cells/µL or x10(3)/mcL
Differential Counts	%
Reticulocyte	%

10.4 Clinically Reportable Range (CRR)

Parameter	Clinical Reportable Range
WBC	0-800 x 10^3
RBC	0-16.00 x 10^6
HGB	0-25
HCT	Calculated and limited by direct measurement reportable ranges
MCV	0-150
MCH	Calculated and limited by direct measurement reportable ranges
MCHC	Calculated and limited by direct measurement reportable ranges
PLT	0-3,000 x 10^3
% NEUTS	0-100
% LYMPHS	0-100
% MONO	0-100
% EOS	0-100
% BASO	0-100
Retic, automated	0.0-30.0%

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10.5 Repeat Criteria and Resulting

Refer to Addendum 2

Parameter	Repeat Tolerance Limits
WBC	± 0.8
RBC	± 0.25
HGB	± 0.6
HCT	± 1.7
MCV	± 3.0
MCH	± 1.2
MCHC	± 1.2
PLT	± 10%
NE%	± 5.0
LY%	± 5.0
MO%	± 3.0
EO%	± 2.0
BA%	± 1.0

11. EXPECTED VALUES

11.1 Reference Ranges

Refer to Addendum 1

11.2 Critical Values

Parameter	Age	Critical Low	Critical High	Reference Units
HGB	1 month and older	≤ 6.0	≥ 20.0	g/dL
HGB	0-29 days	≤ 6.0	≥ 24.0	g/dL
WBC	all ages	≤ 2.0	≥ 30.0	x10(3)/mcL
Platelet	all ages	≤ 30	≥ 900	x10(3)/mcL

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

- **CBC –** The quantitative and qualitative analysis of the cellular elements of blood will identify imbalance between cell production, cell release, cell survival, or cell loss. This information increases the accuracy and specificity of diagnosis based on pathogenesis and is also used to monitor the effectiveness of therapy.
- **Automated Differential –** The Differential distribution of white blood cells will, when correlated with absolute white cell count, identify imbalances between cell production, cell release, cell survival and/or cell loss. This information increases the accuracy and

specificity of diagnosis based on pathogenesis and is also used to monitor the effectiveness of therapy.

- **Platelet Count –** Platelets must be present in adequate numbers and have proper function to aid in hemostasis. A normal bleeding time is dependent on adequate platelet number and function.
- **Reticulocyte Count -** The enumeration of reticulocytes provides an effective means of determining red cell production and regeneration. Elevation is seen in patients with hemolytic anemia, hemorrhage (acute and chronic), treatment of iron-deficiency anemia and megaloblastic anemias and uremia. Decreased counts may be seen in aplastic anemia, aplastic crisis of hemolytic anemias and ineffective erythropoiesis as seen in thalassemia, pernicious anemia and sideroblastic anemia.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

13.1 Manual versus Automated Differential Counts – 95% Comparison Confidence Limits

- This table can be used for two purposes:
- To show the tolerance limits of a manual differential at various levels of counting *(100-cell diff, 200-cell diff, etc.)*
- To determine the tolerance allowed for a technologist performing a 100-cell diff to verify an automated differential with a 95% confidence limit. *(If the instrument reports 20% monocytes, the technologist would be expected to find 12-30% monocytes in the100-cell differential count in order to verify the instrument count.)*
- "A" is the percentage of cell type counted, e.g. lymphocytes.
- "N" is the size of the manual differential performed.

A = % of a cell type	N = 100	N = 200	N = 500	N = 1000
0	0 - 4	0 - 2	0 - 1	0 - 1
1	0 - 8	0 - 4	0 - 3	0 - 2
2	0 - 8	0 - 6	0 - 4	1 - 4
3	0 - 9	1 - 7	1 - 5	2 - 5
4	1 - 10	1 - 8	2 - 7	2 - 6
5	1 - 12	2 - 10	3 - 8	3 - 7
6	2 - 13	3 - 11	4 - 9	4 - 8
7	2 - 14	3 - 12	4 - 10	5 - 9
8	3 - 16	4 - 13	5 - 11	6 - 10
9	4 - 17	5 - 14	6 - 12	7 - 11
10	4 - 18	6 - 16	7 - 13	8 - 13
15	8 - 24	10 - 21	11 - 19	12 - 18
20	12 - 30	14 - 27	16 - 24	17 - 23
25	16 - 35	19 - 32	21 - 30	22 - 28

A = % of a cell type	N = 100	N = 200	N = 500	N = 1000
30	21 - 40	23 - 37	26 - 35	27 - 33
35	25 - 46	28 - 43	30 - 40	32 - 39
40	30 - 51	33 - 48	35 - 45	36 - 44
45	35 - 56	37 - 53	40 - 50	21 - 49
50	39 - 61	42 - 58	45 - 55	46 - 54
55	44 - 65	47 - 63	50 - 60	51 - 59
60	49 - 70	52- 67	55 - 65	56 - 64
65	54 - 75	57 - 72	60 - 70	61 - 68
70	60 - 79	63 - 77	65 - 74	67 - 73
75	65 - 84	68 - 81	70 - 79	72 - 78
80	70 - 88	73 - 86	76 - 84	77 - 83
85	76 - 92	79 - 90	81 - 89	82 - 88
90	82 - 96	84 - 94	87 - 93	87 - 92
91	83 - 96	86 - 95	88 - 94	89 - 93
92	84 - 97	87 - 96	89 - 95	90 - 94
93	86 - 98	88 - 97	90 - 96	91 - 95
94	87 - 98	89 - 99	91 - 96	92 - 96
95	88 - 99	90 - 98	92 - 97	93 - 97
96	90 - 99	92 - 99	93 - 98	94 - 98
97	91 - 100	93 - 99	95 - 99	95 - 98
98	92 - 100	94 - 100	96 - 100	98 - 99
99	94 - 100	96 - 100	97 - 100	98 - 100
100	96 - 100	98 - 100	99 - 100	99 - 100

13.2 WBC Estimate

IF	Then
Using the 50X objective	Calculate the average WBC in 10 fields. Multiply by 3,000.
In the presence of a cellular interference flag perform a WBC estimate. If WBC estimate does not equal the Coulter WBC within ±20%	Investigate the cause. Poor area on smear chosen to do estimate - repeat the estimate. Platelet clumps present – remove the PLT Count and add CLMP to the report NRBCs and/or megakaryocytes or giant platelets present - correct the WBC. From the Histogram keyboard enter the UWBC count. Perform the manual diff (refer to Addendum 8) and correct the WBC. No apparent cause - Have the test redrawn.

13.3 Platelet Estimate

IF	Then
In the presence of a platelet flag, a platelet estimate must be performed. Using the 100X objective	Count the PLT in each of 10 microscopic fields in areas of the slide where the RBCs are evenly dispersed. Divide the total # of platelets by 10 to establish the mean and multiply by 20,000.
The Coulter platelet count and the platelet estimate do not agree within ± 20%	Repeat the platelet estimate and/or platelet count. If counts still do not agree, consult the supervisor or designee.

13.4 RBC Morphology

- Microcytosis, Macrocytosis and Anisocytosis will be quantitated using the LH criteria. The morphology will be quantitated by smear evaluation.
- All clinically significant findings such as specific cell types, inclusions, polychromasia, etc., will be reported from the smear evaluation.

IF	Then
NO clinically significant findings to be added to a patient report.	Result as Normal.
ANY additions to the patient report, such as RBC morphology, cell differential, PLT morphology, etc.	Report all clinically significant findings using the Diff key board in the LIS.

- For consistent morphological reporting, the following criteria are recommended. They serve only as a guideline for evaluating slight, moderate, or marked degrees of abnormal morphology.

Variation	Mean Range per 10 Fields (100x) of RBCs	Then
Poikilocytosis	0	Normal
	1-5	1+
	6-15	2+
	Over 15	3+
Anisocytosis	0-5	Normal
	6-15	1+
	15-30	2+
	Over 30	3+
Polychromasia	0-2	Normal
	3-4	1+
	5 - 6	2+
	Over 6	3+
Hypochromia	0-5	Normal
	6-15	1+
	16-30	2+
	Over 30	3+

Listed below is a guideline for abnormal shapes based on mean range/10 fields of RBCs.

Abnormal Shape	Normal	1+	2+	3+
Spherocyte, Acanthocyte Sickle cell, Rouleaux	0	1-5	6-15	Over 15
Helmet cell	0-1	1-5	6-15	Over 15
Tear drop, Target cell, Schistocyte, Ovalocyte, Elliptocyte, Burr cell, Stomatocyte, Blister cell	0-1	2-5	6-15	Over 15

13.5 Potential Causes of Erroneous Results with Automated Cell Counter

Parameter	Causes of Spurious Increase	Causes of Spurious Decrease
WBC	Cryoglobulin, Cryofibrinogen, Heparin, Monoclonal Proteins, Nucleated RBC, PLT Clumps, Lyse-resistant RBC *NOTE: The LH is able to "gate-out" interferences <35fL in size and provides a "Corrected WBC." The "uncorrected WBC" is available in the comment field for purposes of review. In the absence of a "Cellular Interference" flag, Beckman Coulter believes the WBC to be correct - however, in the presence of interferences WBC values should always be compared to WBC estimates*	Clotting, Smudge Cells, Uremia, Immunosuppressants
RBC	Cryoglobulin, Cryofibrinogen, Giant PLTs, High WBC *(>50,000/µL)*	Auto-agglutination, Clotting, *in vitro* Hemolysis, Microcytic RBC
Hemoglobin	Carboxyhemoglobin *(>10%),* Cryoglobulin, Cryofibrinogen, *in vitro* Hemolysis, Heparin, High WBC *(>50,000/µL),* Hyperbilirubinemia, Lipemia, Monoclonal Proteins	Clotting, Sulfhemoglobin
Hematocrit *(Automated)*	Cryoglobulin, Cryofibrinogen, Giant PLTs, High WBC *(>50,000/µL),* Hyperglycemia *(Glucose >600 mg/dL)*	Autoagglutination, Clotting, *in vitro* Hemolysis, Microcytic RBC
MCV	Cryofibrinogen, Autoagglutination, High WBC (>50,000/µL), Hyperglycemia, Reduced RBC Deformability	Cryoglobulin, Giant Platelets, *in vitro* Hemolysis, Microcytic RBC, Swollen RBC
MCH	High WBC *(>50,000/µL),* Spuriously High HGB, Spuriously Low RBC	Spuriously Low HGB, Spuriously High RBC
MCHC	Auto-agglutination, Clotting, Lipemia, *in vitro* Hemolysis, Spuriously High HGB, Spuriously Low HCT	High WBC *(>50,000/µL),* Spuriously Low HGB, Spuriously High HCT

Parameter	Causes of Spurious Increase	Causes of Spurious Decrease
Platelets	Cryoglobulin, Cryofibrinogen, Hemolysis *(in vitro and in vivo)*, Microcytic RBC, RBC Inclusions, WBC Fragments	Clotting, Giant PLT, Heparin, PLT Clumping, PLT Satellitosis

13.6 Platelet Clumps

Platelet clumping represents agglutination rather than aggregation, as it is not prevented by inhibitors of the platelet release reaction. In addition to pseudo- thrombocytopenia, platelet agglutination may cause pseudoleukocytosis due to the counting of platelet clumps as leukocytes by automated analyzers. Thus, resolving the PLT clumping when possible improves the quality of result provided to the clinician.
When the platelet clump flag is noted check the specimen for a clots and fibrin.
Vortex the EDTA specimen for 1-2 minutes, then rerun the specimen.
If no clumps are seen following vortexing and the platelet count has increased, the count may be reported. However, exercise caution in the situation when only partial resolution of clumping is observed, even if the platelet count increases substantially.
If the post-vortex PLT count is normal, enter a comment that platelet clumping is present but the platelet count is adequate.

If	Then
If PLT count ≤ 130 with significant PLT clumps found during slide scan.	Remove the PLT count number and result with the comment CLMP = *Clumped platelet*

13.7 Sodium Citrate for Platelet Count

Collection of a platelet count with Sodium Citrate anticoagulant is usually reserved for patients who are known to have a platelet clumping phenomena associated with EDTA anticoagulant. The specimen of choice is both an EDTA and a sodium citrate tube. The EDTA is used for the CBC results. The sodium citrate tube is used for the citrate Platelet count. Run samples as per the LH750 protocol. Multiply the Na citrate platelet count by 1.1 to correct for dilution effects.

13.8 MCHCs greater than 36.5 or less than 29.0

If the MCHC is ≤ 29.0 or ≥ 36.5, it should be repeated on the LH750 to rule out random error. If MCHC is ≤ 29.0 a slide should be made and scanned to look for potential causes of spuriously low MCHC, i.e. marked sickle cells or target cells. If the MCHC is greater than 36.5, a slide should be made and examined as well as visual inspection of the sample to determine the integrity of the specimen. The smear review/ visual inspection should indicate to the technologist which category the specimen falls into – cold agglutinin, lipemia, hemolysis, icterus or the situation where the results are accurate due to the presence of spherocytes.

<table>
<tr><th>IF</th><th>Then</th></tr>
<tr><td>Spherocytes are noted on the slide scan</td><td>Report the MCHC with a comment reflecting the presence of spherocytes as 1+, 2+ or 3+.</td></tr>
<tr><td>Resistant hemoglobin, marked sickle cells or target cells noted on the slide scan</td><td>Specimens with lyse resistant RBCs should be repeated on dilution using bottled, distilled water. Prepare a 1:2 dilution with equal parts of blood and water. Allow to sit three minutes. Resuspend and process through the analyzer. Using the HGB result, multiply the results by 2 to determine the corrected hemoglobin result. Use the corrected HGB to recalculate the MCH and the MCHC.</td></tr>
<tr><td rowspan="3">If significant RBC clumping is noted on the slide scan.</td><td colspan="2">Warm specimen in a 37°C water bath or heat block for 30 minutes and rerun. If not resolved, continue warming and rerun every 15 minutes continuing incubation after each run, not to exceed one hour. If necessary, make a warmed slide for morphology evaluation</td></tr>
<tr><td>IF After Incubation</td><td>Then</td></tr>
<tr><td>The MCHC is within normal range</td><td>Report results with the appropriate comment: Specimen was prewarmed to 37ºC to obtain results; Cold agglutinin/cryoglobulin suspected.</td></tr>
<tr><td></td><td>The MCHC is still outside 36.5 after 1 hour incubation: (irreversible cold agglutinins)</td><td>Perform Plasma Replacement Procedure: See Addendum 6.</td></tr>
<tr><td>If hemolysis is suspected on the slide scan, i.e. schistocytes</td><td colspan="2">Examine the specimen for visual hemolysis. If gross hemolysis is observed, cancel the specimen with the appropriate comment: -HMT</td></tr>
<tr><td>If lipemia or icterus is suspected on the slide scan.</td><td colspan="2">Examine the specimen for visual lipemia /icterus. If observed perform a plasma hemoglobin blank. If there is sufficient specimen, mix well and pour off a portion into a plastic specimen tube. Spin the tube for 5-10 minutes at 2000 rpm. If the specimen is short, spin the lavender tube for 5-10 minutes at 2000 rpm. In secondary mode run LH Series Diluent as a blank. Verify a “0” hemoglobin value. In the secondary mode, aspirate plasma portion of spun specimen to determine the plasma hemoglobin blank value. Using the following formula:
Correct Hgb = OH – [PB x (1 – HCT/100)]
Where OH = original hemoglobin
PB = plasma hemoglobin blank
HCT = original hematocrit
Calculate corrected HGB. Enter the corrected HGB on the report and recalculate the indices (formula in addendum #5) and enter the correct results with the comment**:** *“Results were obtained by repeat analysis to include running a plasma blank to eliminate interferences caused by either WBCs, lipemia, or protein entities.”*</td></tr>
</table>

13.9 Correction for Nucleated RBCs and/or Megakaryocytes and/or when a Cellular Interference flag is received.

1. Whenever the LH instrument gives a cellular interference flag, a slide WBC estimate has to be done. See section 13.2. If the estimate does not match within 20% of the LH WBC count a WBC correction has to be done. Use the following calculation if this correction has to be done manually.

   The LH750 reported WBC is always "corrected" for presence of interfering substances <35 fL in size. The "uncorrected" WBC is available in the COMMENT field of the instrument print-out for review, if necessary. The instrument "corrected" value is the value reported in LIS by the instrument. If slide review indicates presence of **>10nRBCs** or megakaryocytes, the uncorrected WBC count must be used in the calculation to avoid overcorrection. Use LIS code **WNRBC** to append the following message to the WBC result: White blood cell count corrected for presence of nucleated red blood cells.

   $$\text{Corrected WBC} = \frac{\textbf{Uncorrected WBC} \times 100}{100 + \#\text{NRBC's and/or megakaryocytes}}$$

2. Whenever the LH instrument enumerates **NRBC'S >5** a slide MUST be reviewed for the presence of NRBC. If no NRBC is seen on the smear, the Coulter LH NRBC count should be removed.

13.10 Slide Preparation

When making a smear always check the specimen for clots. This can be done by visual inspection or by the use of an applicator stick when appropriate. Refer to Addendum 8 for smear preparation.

13.11 Coulter Repeats

(*See Addendum 2*) Results must be reported with the comment.
REP = *RESULTS CONFIRMED, TEST REPEATED.*

13.12 SCAN Smear

Refer to Addendum 8 for Scan instructions.

13.13 Correction of RBC & HGB in the presence of a WBC count greater than 400,000.

a) Subtract the WBC count from the RBC count to obtain a corrected RBC Count.
b) Spin an aliquot of specimen for 3-5 minutes at 1500 rpm.
c) Remove an aliquot of the red cell portion, and dilute it 1:2 with LH Series Diluent.

d) Run the "RBC only" suspension as a sample on the Coulter to obtain an accurate MCH and MCV.
e) Calculate the corrected HGB: Hgb = MCH x RBC (corrected) / 10
f) Calculate the HCT: Hct = MCV x RBC (corrected) / 10
g) Calculate the MCHC: $\text{MCHC} = \frac{\text{Corrected Hgb}}{\text{Corrected Hct}} \times 100$

13.14 Special Reticulocyte Precautions

Specimens with verify retic flag other than those listed in addendum 2 must be verified by a manual reticulocyte count. Refer the sample to the reference laboratory.

13.15 Dilutions

The following table lists the maximum dilutions necessary to comply with the **CRR** (See Section 10.4 for CRR values).

- Dilutions should be made with LH Series Diluent.
- For results greater than the CRR, report according to the chart.

Parameter	Dilutions for CRR	
	LH750	Report as…
WBC	1:2	>800 x 10^3
RBC	1:2	>16.00 x 10^6
HGB	none	NA
PLT	none	NA

13.16 Alternative Procedures

None

14. LIMITATIONS OF METHOD

14.1 CBC-Line Linearity

- CBC-Line Linearity is an assayed material used to establish the Analytical Measurement Range and verify the calibration of the LH750. This product allows Quest Diagnostics to comply with CLIA 88 Regulations and CAP requirements.
- Analytical Measurement ranges should be performed at installation and when necessary to verify linearity.

Recommended Linearity Kit	Supplier
Beckman Coulter Lin-C Linearity Kit (Follow manufacturers requirements for storage and stability)	Beckman Coulter

14.2 Analytical Measurement Range (AMR)

Parameter	Analytical Measurement Range
	LH750
WBC	0-400 x 10^3
RBC	0-8.00 x 10^6
HGB	0-25
MCV	0-150
Reticulocyte	0.0 – 30.0 %
PLT	0-3,000 x 10^3

14.3 Precision

Recovered in the procedure validation package

Inter-Run				
Analyte	**Level**	**Mean**	**1SD**	**CV%**
WBC	LOW	1.15	0.08	6.90
	MID	20.01	0.18	0.90
	HIGH	94.57	1.28	1.36
PLATELET	LOW	4.82	0.48	10.06
	HIGH	647.29	20.69	3.20
HEMOGLOBIN	LOW	5.16	0.08	1.53
	HIGH	16.76	0.13	0.76

Intra-Run			
Analyte	**Mean**	**1SD**	**CV%**
WBC	7.40	0.10	1.33
RBC	4.87	0.03	0.62
HEMOGLOBIN	15.21	0.09	0.62
PLATELET	235.86	8.15	3.46
% NEUTS	63.85	0.42	0.33
% LYMPHS	27.30	0.43	1.57

14.4 Interfering Substances

See 13.5

14.5 Clinical Sensitivity/Specificity/Predictive Values

Not applicable.

15. SAFETY

You, the employee, have a direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

16. RELATED DOCUMENTS

- Material Safety Data Sheets
- LH 750 Reference Manual
- Critical Values (Lab policy)
- Quality Control Program policy
- CUM or ICUM, LIS procedure
- Quest Diagnostics Records Management Program
- Laboratory Safety Manual
- Current package inserts for Coulter® S-Cal® Calibrator Kit Package, Coulter® Latron 1 and 2, Coulter® 5C® Cell Control, and Coulter® Retic-C
- Pathologist Slide Review Request (AG.F127)
- LH 750 Maintenance Log (AG.F257)
- Daily Quality Control Schedule for LH750, GEC (AG.F273)

17. REFERENCES

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3. "Differential Leukocyte Counting", CAP Conference, Aspen 1977, John A. Koepke, M.D., Ed., Published by College of American Pathologists.
4. Color Atlas of Hematology CAP Hematology and Clinical Microscopy Resource Committee Distinction between megakaryocyte and giant platelets in the section "Megakaryocyte Maturation".

5. Hematology: Principles and Procedures by Barbara A. Brown, 2nd Edition, 1976, pp 77-81.
6. A Color Atlas & Instruction Manual of Peripheral Blood Cell Morphology, O'Connor Bartera, 1984.
7. Nathan, David G. & Oski, Frank A. "Hematology of Infancy and Childhood", 3rd edition, 1987.
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9. Miller, Dennis R. Ed. "Blood Diseases of Infancy and Childhood", 5th edition, 1984.
10. Henry, J B, ed. "Clinical Diagnosis and Management by Laboratory Methods", 20th edition, 2001.
11. Coulter® S-Cal® Calibrator Kit Package insert, Beckman Coulter, 2009.
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13. Coulter® 5C® Cell Control Package insert, Beckman Coulter, 2008.
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15. Gulati GL, Asselta A, Chen C. Using a vortex to disaggregate platelet clumps. Laboratory Medicine. 1997;28:665-667.
16. Rodak B, Fritsma G, & Doig, K. "Hematology: Clinical principles and Applications", Third edition, 2007, pg 176-177 and 526-530.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
	8/6/09		Supersedes SOP WAH-SGAH H001.002	C. Reidenauer	Dr. Cacciabeve
000	6/25/10	5.3 8.6 9.0 11.2 13.2 13.6 13.12 16 Addenda 2&3	Cal Freq. and Pre-calibration instructions Added Path Review Instructions Slight edit to Calculations statement Update terminology CLMP – added Remove the PLT Count Addition of vortexing to remove EDTA induced platelet clumps. SCAN Smear instructions added Add current package inserts Updates to the differential and smear review criteria. Updated owner	R. SanLuis	Dr. Cacciabeve
001	7/20/11	3.2 5.3 6.2 6.3D 13.12 15 17 19 Addenda 2	Remove tube sizes Cal Freq. changed to at least 6 months Follow QC Program, revise 5C sampling to 13 day or 13 times. 5C is depleted in 11-12 runs Content combined with addenda 8 Update to approved format Add Pediatric Hematology, Rodak Add 8 and 9, renumber last addenda Add differential timing, Add action for ABN RETIC pattern on newborns <30	R. SanLuis C. Reidenauer R. SanLuis R. SanLuis R. SanLuis L. Barrett R. SanLuis R. SanLuis R. SanLuis R. SanLuis	Dr. Cacciabeve

Form revised 7/01/01

Version	Date	Section	Reason	Reviser	Approval
			days. (not addressed in previous versions)		
002	9/17/12	Title page 13.9 13.14 Addenda 2 19	Update owner Add LIS code WNRBC Refer manual reticulocyte counts to reference lab. Actions for reticulocyte flagging Add Quick Reference Chart	R. SanLuis	Dr. Cacciabeve
003	3/10/14	4.3 16 Addenda 7 Addenda 11 Footer	Edit reference to Addenda 7 Add forms Replace form with process steps Pathologist Slide Review Request moved to section 6 Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	C. Reidenauer L. Barrett	Dr. Cacciabeve
4	9/18/14	10.3,11.2, Addenda 1	Replaced $10^3/\mu L$ units with x10(3)/mcL	M. Sabonis	Dr. Cacciabeve

19. ADDENDA

Addendum	Title
1	Reference Ranges
2	CBC Diff/Scan Action and Repeat Criteria
3	LH 750 Decision Rules, Flags and Action Criteria
4	Daily quality control for LH750 at SGAH and WAH
5	Calculation Formulas
6	Plasma Replacement
7	Reagent Change and Background Check Process
8	Manual Differential
9	DIFF Keyboard: Accessing Differential Result Entry
10	Quick Reference Differential Flagging Criteria Chart

Form revised 7/01/01

ADDENDUM 1

ADULT CBC AND DIFFERENTIAL REFERENCE RANGES

Male Reference Ranges			Female Reference Ranges	
Parameter/Units of measurement	**13y- 19y**	**> 19 years**	**13y – 19y**	**> 19 years**
WBC/ x10(3)/mcL	4.5 – 13.0	4.5 – 11.0	4.5 – 13.0	4.5 – 11.0
RBC/ 10^6/µL	4.5 – 5.3	4.5 – 6.3	4.1 – 5.1	3.9 – 5.6
HGB/ g/dL	13.0 – 16.0	13.5 – 18.0	12.0 – 16.0	11.5 – 16.0
HCT/ %	37.0 – 49.0	39.0 – 52.0	36.0 – 46.0	33.0 – 47.0
MCV/ fL	78 - 102	80 - 100	78 - 102	76 – 101
MCH/ pg	25.0 – 35.0	26.0 – 36.0	25.0 – 35.0	26.0 – 36.0
MCHC/ g/dL	32.0 – 37.0	32.0 – 37.0	32.0 – 37.0	32.0 – 37.0
RDW/ %	11.5 – 14.0	11.5 – 14.0	11.5 – 14.0	11.5 – 14.0
PLT/ x10(3)/mcL	150 - 450	150 - 450	150 - 450	150 - 450
MPV/ fL	7.2 – 11.1	7.2 – 11.1	7.2 – 11.1	7.2 – 11.1
Absolute Neutrophils/ x10(3)/mcL	2.10 – 11.52	1.89 – 7.92	2.10 – 11.52	1.89 – 7.92
Absolute Lymphs/ x10(3)/mcL	0.77 – 5.85	0.77 – 4.95	0.77 – 5.85	0.77 – 4.95
Absolute Monocytes/ x10(3)/mcL	0.14 – 1.30	0.14 – 1.10	0.14 – 1.30	0.14 – 1.10
Absolute Eosinophils/ x10(3)/mcL	0 – 0.78	0 –0.66	0 – 0. 78	0 – 0.66
Absolute Basophils/ x10(3)/mcL	0 – 0.26	0 –0. 22	0 – 0.26	0 – 0.22
Nucleated RBC/ 100 WBC	0	0	0	0
Retic – Automated %	0.6-2.7	0.6-2.7	0.6-2.7	0.6-2.7
Retic – Manual %	0.5-1.5	0.5-1.5	0.5-1.5	0.5-1.5

PEDIATRIC CBC AND DIFFERENTIAL REFERENCE RANGES

Parameter/Units of Measurement	0d	2d	3d	2w	1m	2m	3m	6m	1y	2y	6y – 12y
WBC/ x10(3)/mcL	19.0–25.0	9.0-30.0	9.0-30.0	9.0-30.0	5.0-19.5	5.0-19.5	5.0-19.5	6.0-17.5	6.0-17.5	6.0-17.0	5.0-16.0
RBC/ 10^6/µL	4.00-6.60	3.90-5.90	3.90-5.90	3.90-5.90	3.10-5.30	3.10-5.30	2.70-4.50	3.10-5.10	3.90-5.50	3.90-5.50	3.90-5.50
HGB/ g/dL	14.5-22.0	13.4-19.9	13.4-19.9	13.4-19.9	10.7-17.1	9.1-14.0	9.1-14.1	9.5-14.1	11.3-14.1	11.3-14.1	11.5-14.0
HCT/ %	45.0-65.0	42.0-65.0	42.0-65.0	42.0-65.0	33.0-55.0	28.0-42.0	29.0-41.0	29.0-41.0	31.0-41.0	31.0-41.0	34.0-42.0
MCV/ fL	95.0-121.0	88.0-123.0	88.0-123.0	88.0-123.0	88.0-123.0	91.0-112.0	74.0-108.0	74.0-108.0	70.0-86.0	70.0-86.0	73.0-87.0
MCH/ pg	31.0-37.0	31.0-37.0	31.0-37.0	31.0-37.0	27.0-36.0	27.0-36.0	25.0-35.0	25.0-35.0	23.0-31.0	23.0-31.0	24.0-30.0
MCHC/ g/dL	29.0-37.0	28.0-36.0	28.0-36.0	28.0-36.0	28.0-36.0	28.0-36.0	28.0-36.0	30.0-36.0	30.0-36.0	30.0-36.0	31.0-36.0
RDW/ %	11.5-14.0	13.0-18.0	13.0-18.0	11.5-16.0	11.5-16.0	11.5-16.0	11.5-16.0	11.5-16.0	11.0-15.0	11.0-15.0	11.0-15.0
PLT/ x10(3)/mcL	150-450	150-450	150-450	150-400	150-400	150-400	150-400	150-400	140-400	140-400	140-400
MPV/ fL	7.2-11.0	7.5-11.6	7.5-11.6	7.5-11.6	7.5-11.6	7.5-11.6	7.5-11.6	7.5-11.6	7.5-11.6	7.5-11.5	7.5-11.5
Absolute Neutrophils/ x10(3)/mcL	11.59-18.75	5.31-21.90	4.41-18.30	2.97-12.30	1.50-7.02	1. 50-7.02	1.65-8.00	1.98-7.18	1.98-7.18	2.22-7.65	2.10-11.52
Absolute Lymphs/ x10(3)/mcL	3.61-5.75	2.43-11.40	2.61-12.00	4.68-20.70	2.75-14.04	2.75-14.04	2.80-12.29	3.30-12.60	2.88-11.03	2.88-10.71	1.75-7.68
Absolute Monocytes/ x10(3)/mcL	0.95-3.75	0.00-1.50	0.36-2.40	0.18-1.80	0.10-1.17	0.15-1.95	0.15-1.95	0.18-1.75	0.18-1.75	0.18-1.70	0.15-1.60
Absolute Eosinophils/ x10(3)/mcL	0-1.50	0.00-1.80	0.00-1.80	0.00-1.80	0.00-1.17	0.00-1.17	0.00-1.17	0.00-1.05	0.00-1.05	0.00-1.02	0.00-0.96
Absolute Basophils/ x10(3)/mcL	0-0.50	0.00-0.60	0-0.60	0-0.60	0.0-0.39	0.0-0.39	0.0-0.39	0.0-0.35	0.0-0.35	0.0-0.34	0.0-0.32
Nucleated RBC/ 100 WBC	0	0	0-8	0	0	0	0	0	0	0	0
Retic- Auto %	1.0-7.5	1.0-7.5	1.0-7.5	0.6-2.7	0.6-2.7	0.6-2.7	0.6-2.7	0.6-2.7	0.6-2.7	0.6-2.7	0.6-2.7
Retic – Manual %	0.5-4.5	0.1-1.5	0.1-1.5	0.1-1.5	0.1-1.5	0.1-1.5	0.1-1.5	0.1-1.5	0.1-1.5	0.1-1.5	0.1-1.5

The reference ranges should be interpreted as from and including the age specified in the title of the column

Pediatric and Adult % Differential Reference Ranges

Parameter/Units of Measurement	0d	1d	2d	3d	4d	6d	8d	15d	1m	2m	3m	4m	7m	1y	13m	3y	4y	5y	6y	7y	12y+
Neutrophils/ %	61 - 75	60 -74	59-73	49-61	45-55	37-45	33-41	29-35	30-36	30-36	33-41	33-41	35-43	35-43	37-45	39-47	42-52	44-54	42-72	42-72	42-72
Bands / %	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15	1-15
Myelocyte/ %	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metamyelocytes/ %	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1
Promyelocytes/ %	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blast / %	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymphocytes/ %	19-23	22-28	27-33	29-35	32-40	40-48	46-56	52-64	55-67	55-67	56-68	55-67	52-64	48-58	48-58	43-53	42-52	39-47	35-43	34-42	17-45
Atypical Lymphocytes / %	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5
Monocyte / %	5-15	5-15	4-8	4-8	4-8	4-8	4-8	2-6	2-6	3-10	3-10	3-10	3-10	3-10	3-10	3-10	3-10	3-10	3-10	3-10	3-10
Basophil/ %	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2
Eosonophil/ %	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6
Plasma Cell/ %	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1

Reference: % Cell Differential were obtained from Sunquest Quality Assurance's Database

ADDENDUM 2

CBC DIFF/SCAN ACTION AND REPEAT CRITERIA

KEY	
RPT –	repeat CBC on LH750
SCAN –	microscopically scan smear & manual differential if required (refer to Addendum 8)

Parameter	Condition		Action Needed
WBC	≤ 2.0	DIFF	• Re-analyze, verify count within ± 15% Add the comment that the result was checked. • Check sample for clots. If clotted, cancel the test and notify the ordering doctor or unit. • If unable to evaluate 100 cells, do a 50 cell diff and multiply the results by 2. • Re-analyze, scan to verify count verify count within ± 15%. Add the comment that the result was checked . • Excessive number of small WBCs below the 35 fL threshold
	≥ 30.0	SCAN	Scan to verify count. Rule out erroneous increase due to: • 2-3+ presence of large/giant platelets. Add appropriate message code. • Presence of abnormal protein/cryoglobulin *(blue streaks in smear)*. • Presence of NRBC. Correct WBC. Add appropriate message code. • Presence of unlysed particles above WBC threshold of 35 fL *(crystals, lyse-resistant RBC)*.
	≥ 400.0	RPT by Dilution	• Re-analyze by dilution. Refer to AMR limits. Add comment RESULTS VERIFIED BY REPEAT ANALYSIS. • Refer to WBC ≥ 30.0
	WBC R or * flag, an NRBC flag, or Cellular Interference	SCAN	• Insure the specimen is adequately mixed. Vortex for 1-2 minutes and repeat. If resolved release results else hold for slide review. • Scan to verify WBC estimate. • Rule out erroneous results due to the presence of NRBC, PLT clumps or giant PLTs.
RBC	*≥ 8.00* RBC Morphology Flag	RPT by Dilution, MORPH	• Re-analyze by dilution. Refer to AMR limits. Add comment REP = *RESULTS CONFIRMED, TEST REPEATED* • Scan to verify morphology. Report morphology.
HGB	≤ 6.0	RPT MORPH	• Re-analyze, verify count. Add comment. • Check for good H&H match. • Check sample for clots.
	≥ 20.0 (excludes neonates) ≥ 25.0 *(AMR)*	RPT MORPH	• Re-analyze if greater than 20.0. Add comment. • **If greater than 25.0 repeat by dilution.** • Rule out hemoconcentration. (pour off) • Check age of patient. • Check coagulation sample if HCT ≥ 55.0

Parameter	Condition		Action Needed
			Note: Another quick check is to view the clot tubes on the patient for visibly high HCT level.
MCV	≤ 50.0	RPT MORPH	• Verify by repeat analysis. Add comment. • Verify value consistent with morphology review. See Action Needed on <70.0 MCV.
	< 70.0	MORPH	• Verify value consistent with morphology review. Denote any Target Cells, Sickle Cells, Schistocytes or Spherocytes. For 2+ or greater RBCs below threshold, evaluate accuracy of RBC count, consult supervisor if necessary.
	> 110	MORPH	• Verify that value is consistent with morphology review.
	≥ 130.0	MORPH	• Verify that value is consistent with morphology review. • Denote any rouleaux or RBC agglutinins, apply message codes, and consider holding quantitative values. If necessary, consult supervisor. • Pathologic conditions include macrocytic anemias such as pernicious anemia *(oval macrocytes with hypersegmented neutrophils)* and other megaloblastic anemia. • Check for presence of cold agglutinins or cryoglobulins. Usually see elevation of MCHC also. Warm specimen to 37°C, 30 minutes and retest. • Apply message codes.
MCHC	≥ 36.5 ≤ 29.0	RPT *(warmed)* SCAN	Refer to Section 13.8.
RDW	> 21.0	Scan	• Verify value consistent with morphology review. • Review smear for RBC abnormalities.
Platelet	< 50	RPT, Check for clot, Perform PLT EST	• Verify by repeat analysis. Add comment • Be suspicious if occasional fields on morphology review have 2-3 platelets/hpf. Check closely for fibrin, >2+ large/giant platelets, platelet satellitism or platelet clumps. Check tube for clot. Scan the feather edge of the smear.
	> 50 and < 100 No flags & No History	Perform PLT EST	• *Review smear for large PLTs to ensure there is not a PLT gating (size classification) error with no previous history.*
	Platelet Clumped suspect flag	Check for clot, Vortex, WBC EST	• *Vortex specimen for 1-2 min and repeat. Refer to section 13.6.* • Perform scan to rule out interferences caused by ≥ 2+ large or giant platelets, plt clumps, platelet satellitism, fibrin, NRBCs, RBC fragments, or old blood/ excessive degeneration, WBC fragments or clumps. • Re-result as in section 13.6 if significant platelet clumping is noted. *Remove the PLT count before the hemogram is released*

Parameter	Condition		Action Needed
Platelet (cont'd)	*≥ 3000*	RPT by dilution, SCAN	• Re-analyze by manual dilution with Coulter Diluent. Correlate with morphology review. Add comment REP = *RESULTS CONFIRMED, TEST REPEATED*
	Other Platelet Flags	PLT EST	Scan diff to verify flag. Report noted observation.
Lymphocytes	≥ 70%	SCAN	Perform scan to rule out immature, variant, atypical or blast cells. *(Refer to Addendum 8)* **Note: For patients <12 years old perform DIFF if the count is inverted.**
Neutrophils	≥ 90%, no flags (including NE1)	SCAN	• Scan to verify neutrophils. Rule out immature neutrophils or precursors. *(Refer to Addendum 8)*
Neutrophils	≥ 90% in the presence of the following flags: **Ig2, blasts, or NRBC**	DIFF	• Perform manual differential
Relative% Monocytes	> 22%	DIFF	Scan to verify monocytes. Rule out immature monocytes or precursors.
Relative % Eosinophils	≥ 25%	SCAN	Scan to verify eosinophils. Rule out presence of parasites.
Relative % Basophils	≥ 3.0%	RPT, SCAN If unresolved	• Ensure specimen is properly mixed. Place on mixer for 5 minutes and repeat. • If suspect artificial increase, retrieve blood and check by repeat analysis, if still elevated, perform manual count. • If smear review reveals increase in basophils, check for leukemic picture.
Reticulocyte	Retic flag	ABN RETIC Pattern, See Action	• If result is within the CRR report automated count. • If result is above the CRR ensure specimen is properly mixed and repeat. • If the result exceeds the CRR on repeat refer to section 10.4. • No repeat required for ABN RETIC pattern on newborns <30 days.
Pediatric Rules	Special "No flag" DIFF Rule	DIFF	• DIFF all patients <1yr • For patients <12 years old perform DIFF if the count is inverted (Lymphocyte% > Neutrophil%) (Patients ≥12 years with no flags as outlined in addendum 2&3 release auto-differential)
Differential Timing	≤ 48 Hours, No change in parameters or improvement	No DIFF, If	• No repeat differential if CBC parameters are improving (moving toward normal) and last differential was performed within 48 hours. (See exceptions below). • Exception 1- Differential required if blasts flagged, • Exception 2- R, *, and cellular interference, see appropriate rule. • Exception 3- Physician request. • Exception 4- Patients < 1 year of age.

ADDENDUM 3

LH 750 Decision Rules, Flags and Action Criteria

Condition (IF)	Action Needed (THEN)
No Match or No Read	Check cassette for sample ID and test request
Any Blasts Flag	Perform Manual Differential
WBC---- or RBC--- or HGB--- or PLT---	Check for clots and Rerun Sample / Troubleshoot
HCT cL or HCT cH or HGB cL or HGB cH	Repeat Sample if no history or delta failure
Imm.NE2 or Variant LY or Low event # or Verify Diff	Perform Manual Differential
NE % --- or LY % --- or Mo % --- or EOS %---	Perform Manual Differential
WBC + or RBC+ or HGB+ or PLT+	Dilute Sample, Repeat, Run Isoton Blank
MPV ----	Type “Hide” in LIS
Part. ASP.	Check for clots and volume of sample, then rerun in manual mode.
Giant Platelets or Platelet Clumps or Thrombocytopenia or PLT R	Vortex 1-2 minutes, Repeat, If unresolved remove PLT result before releasing the Hemogram Review Smear for PLT estimate Refer to Section 13.6
Dimorphic Reds or Red Cell Agglutination	Review Smear for RBC Morphology
Abnormal Retic Pattern	Manual Retic, Utilize rule from addendum 2
NRBC % aH or NRBC % R	Scan Smear for NRBC. If it count does not agree, remove NRBC result before releasing the Hemogram.
Cellular Interference or WBC R R or * Flags	Check for Clots, then Vortex. If not resolved remove WBC and PLT result before releasing the Hemogram. **Scan Smear; Perform a WBC and PLT EST.**
Sys. Alarm	Repeat sample and verify result
H&H Check Failed	Mix sample for 5-10 minutes then repeat. Verify Indices correspond, scan diff if necessary

Note: Refer to Addendum 8 for Manual Differential

ADDENDUM 4

DAILY QUALITY CONTROL FOR LH750 at SGAH and WAH

All controls must be run per shift at the designated time **+/- 30 minutes**.

A. NIGHT SHIFT

2300 Run a 5C Control as bracketing QC on LH1 in closed mode before shutdown. Rotate the level used each day.

2315 Shutdown LH1. Leave in shutdown for 60 minutes.

0015 Startup LH1. If Daily Checks pass, run the Latron primer and Latron control. (See LH help screen for instructions to run Latron.)
Then run all three levels of 5C and Retic controls in closed mode.
File all startup printouts in the maintenance log.

0045 Perform all above steps on LH2.

0430 Run the 5 C Normal controls on both instruments in closed AND open mode.
Run Retic Level I control in open mode on both LH1 and LH2.

B. DAY SHIFT

0730 Run all three levels of 5C and Retic controls in closed mode on both LH1 and LH2.

1130 Run 5 C Abnormal I on LH1 and LH2 in open AND closed mode.
Run Retic Level II control in open mode on LH1 and LH2.

C. EVENING SHIFT

1530 Run all three levels of 5C and Retic controls in closed mode on both LH1 and LH2.

1930 Run 5C Abnormal II on LH1 and LH2 in open AND closed mode.
Run Retic Level III control in open mode on LH1 and LH2.

- **It is critically important that each time any of the levels of the quality controls fail to follow the troubleshooting protocol. When all levels of the quality controls are in range, perform the Patient Look back Procedure.**

- **NEVER report patients when quality controls fail. Call the technical manager if assistance is needed.**

ADDENDUM 5

Calculation Formulas

MCV = (Hct x 10) / RBC

MCH = (Hgb / RBC) x 10

MCHC = (Hgb / Hct) x 100

Absolute neutrophil count = (neutrophil % /100) x WBC

Absolute lymphocyte count = (lymphocyte % /100) x WBC

Absolute monocyte count = (monocyte % /100) x WBC

Absolute eosinophil count = (eosinophil % /100) x WBC

Absolute basophil count = (basophil% / 100) x WBC

ADDENDUM 6

Plasma Replacement with Warm LH Series Diluent

Dispense 5 ml of LH Series Diluent into a plastic tube with a tight fitting lid. Place the tube in a sealed plastic bag and place in the 37°C water bath in blood bank for a minimum of 15 minutes.

Meanwhile, spin a 2 mL aliquot of the patients sample for 10 minutes at 2700 rpm. After spinning, mark the level of the plasma on the outside of the tube. Take off the plasma as far down to the red cells as possible without removing any RBCs.

Fill the tube to the mark with the warmed LH Series Diluent, mix thoroughly and run IMMEDIATELY through the LH750.

Examine the results. If the RBC is within ±0.02 of the original RBC result, the HGB and HCT agree and the MCHC is below 36.5 the results may be reported.

Append the following comment to the RBC result:

37 degree C results due to the presence of a cold agglutinin. Warm diluent replacement performed

ADDENDUM 7

Reagent Change and Background Check Process

Diluent

- Scan in the lot number and expiration date
- Place the new reagent on the instrument
- Program F16 to prime the diluent
- When the instrument is back to **ready** status go to **Main Menu** on the analyzer.
- Choose Analyzer Functions
- Startup Tests
- Background tests
- When complete, initial / date the printout and file

CBC Lyse

- Scan in the lot number and expiration date
- Place the new reagent on the instrument
- Program F02 to prime the CBC Lyse
- When the instrument is back to **ready** status go to **Main Menu** on the analyzer.
- Choose Analyzer Functions
- Startup Tests
- Background tests
- When complete, initial / date the printout and file

Diff Pak and Retic Pak

- Scan in the lot number and expiration date.
- These two reagents do not have prime functions, place the reagent and perform a startup.
- When complete, initial / date the printout and file

Clenz

- Place the reagent, scan in the lot number and expiration date.
- Do a shutdown and a startup.
- When complete, initial / date the printout and file

ADDENDUM 8

SMEAR REVIEW AND MANUAL DIFFERENTIAL

A. General Instruction/Notes
   1. All smears for WBC differential must be made with the wedge pull film technique. A properly made Wright stain blood smear should have the following characteristics:
      a. RBC - Pink with central pallor
      b. NRBC - Dark purple nucleus.
      c. WBC
         - Neutrophil - Dark purple nuclei with light pink cytoplasm dotted with lilac granules.
         - Lymphocyte - Dark purple nucleus. Cytoplasm with varying shades of blue (robin egg blue).
         - Monocyte - Cytoplasm of monocytes stains a faint bluish gray tinge.
         - Eosinophil - The eosinophilic granules, bright red to orange.
         - Basophil - The basophilic granules very dark bluish purple.

   2. Prepare blood films within 4 hours of the blood collection in EDTA.
   3. Stain the film within one hour of preparation with Wright stain.
   4. The leukocytes must be well preserved, and anticoagulant effects such as excessive vacuolization or changes in nuclear shape must be minimal. Less than 2% of the leukocytes may be smudged, except in some lymphoproliferative disorders.
   5. There should be sufficient working area with minimum 2.5 cm in length terminating at least 2 cm from the end of the slide.
   6. Acceptable morphology within working area and no artifact introduced by the technique. Also there should be minimum distributional distortion.
   7. A far end that becomes gradually thinner, without growing streaks, troughs, or ridges, all of which indicate an increased number of leukocytes carried into this area.

B. Prepare a blood smear and Scan:
   1. Transfer a small drop of blood (2-3mm) to a pre-cleaned slide, frosted side up. The blood is placed in the centerline of the slide just past the frosting.
   2. A second slide is used as a spreading slide. The “pusher” slide is placed at an angle of 30-45 degrees to the slide containing the blood drop. The slide is moved back to make contact with the drop, allowing the blood to spread the entire width of the slide.
   3. The “pusher” slide is then quickly and smoothly pushed forward to the end of the smear, creating a wedge smear. It is important that the whole drop is picked up and spread quickly. Moving the pusher slide too slowly accentuates poor leukocyte distribution by pushing larger cells to the very end and the slides of the smear.
   4. The drop of blood should be of such a size that the film is about 30mm in length. (If necessary repeat this process until an acceptable slide is obtained.)
   5. Label the slide using a sharp pencil. On the frosted side, record the patient's last name, or as much as will fit on the slide and accession or barcode. Allow the slide to air dry. Load the slide on the Aerospray slide stainer.
   6. Use the 50x oil immersion objective and examine the smear microscopically. Check the smear to see that it is well made, the distribution of the cells is uniform and the staining is satisfactory.

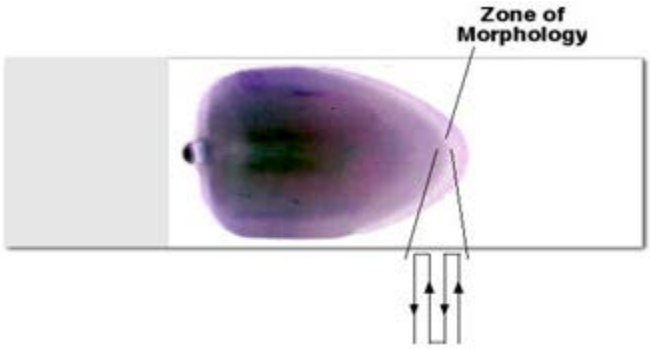
Criteria for a well-stained smear include:

a. No precipitated stain should be seen.
b. The erythrocytes are orange or pink.
c. The nuclei of the leukocytes are purplish-blue.
d. Neutrophilic granules are reddish to pink-Iilac.
e. Eosinophilic granules are red to orange.
f. Basophilic granules are very dark bluish-purple.
g. Platelets stain dark blue-purple.

7. Scan the slide and look for abnormal or suspicious cells that may be in disproportionately low numbers. Look for nucleated red cells, immature cells, atypical lymphocytes and platelet clumps or large platelets. Estimate the white cell count to see if there is any gross error in the instrument count. This could also detect a clotted specimen or perhaps a mix-up in blood specimens.
8. If no significant abnormalities are noted the comment "*Smear review agrees with automated differential. No significant red cell or platelet abnormalities are noted"* should be entered.
9. If a smear review shows a discrepancy with the automated differential or reveals any significant abnormalities or problems for which a manual differential is deemed medically necessary, a manual differential will be performed.

C. Differential Counting Procedure

1. Use the "battlement" tract for this examination. Each identified cell must be classified by cell type. Cells found in a Differential are: neutrophil, segmented; neutrophil, band; lymphocyte, normal; lymphocyte, variant or atypical; monocyte; eosinophil; basophile; other nucleated cells (except nucleated red cells). Include distorted cells that are clearly identifiable in the appropriate classification. While counting the cells, make a note of any abnormalities present in the cells. It is important to examine cellular morphology and to count leukocytes in areas that are neither too thick nor too thin.



2. On each slide, 100 leukocytes should be counted. If the blood is leukopenic, process additional slides in parallel. Exceptions to the 100 cell differential are as follows:

If	Then
WBC greater than 25,000/MM^3	Perform a 100 cell differential count and compare counts to the automated counts. Perform a 200 cells count if difference is greater than 10%.
WBC of greater than 2,000 and less than 25,000	A 100 cell differential count is to be performed
WBC less than 2,000	Perform a 100 cell count if possible; however, a 50 cell differential may be performed, insure correct percentage is entered in LIS.

WBC less than 500	Stain 2-3 slides and perform classifying as many cells as possible, ensure correct percentage is entered in LIS.

When the standard 100 cell Differential is not performed, the number of cells counted must be noted in the LIS.

3. Express the results of the differential count as a percentage of all the leukocytes counted.
4. Count nucleated red blood cells present and report the result as the number per 100 leukocytes counted, see section 13.9.
5. Examine the red cell morphology in a thin area of the slide where the red cells either do not overlap or lightly overlap. They should have a central pallor. In most cases an abnormality must be a consistent finding in order to be significant. Note any variations from normal and classify them according to section 13.4.
6. Examine the smear for platelets morphology and number. Find a thin area where red cells are not overlapping. Perform a PLT estimate, refer to section 13.3.

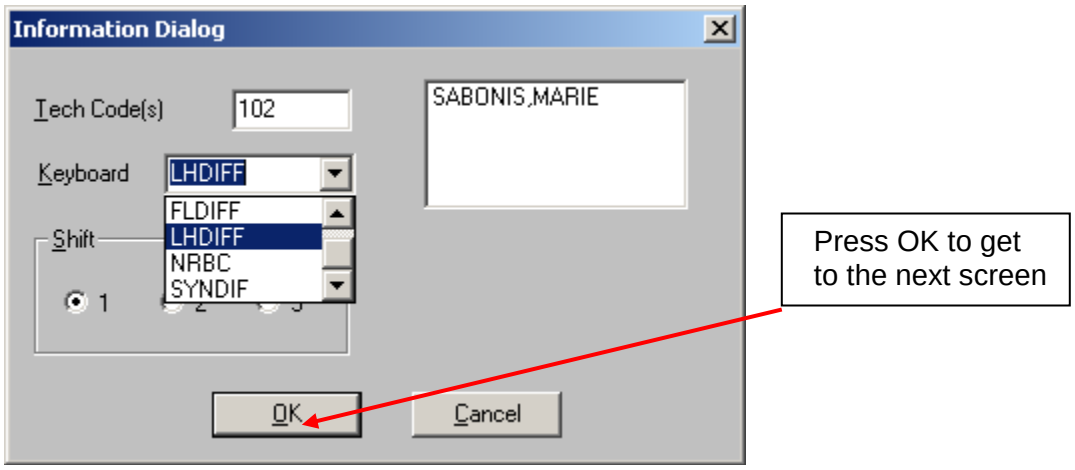
D. **Germantown Emergency Center**: differentials that are to be reviewed by the Pathologist will be sent via courier to Shady Grove Adventist Hospital Hematology section with the required paperwork (refer to section 8.5), who will take the slide and paperwork to the pathologist for review.

NOTE: If malaria is observed in the blood smear: Call the patient's physician and report your finding. The physician may request a malaria smear review and identification.

ADDENDUM 9

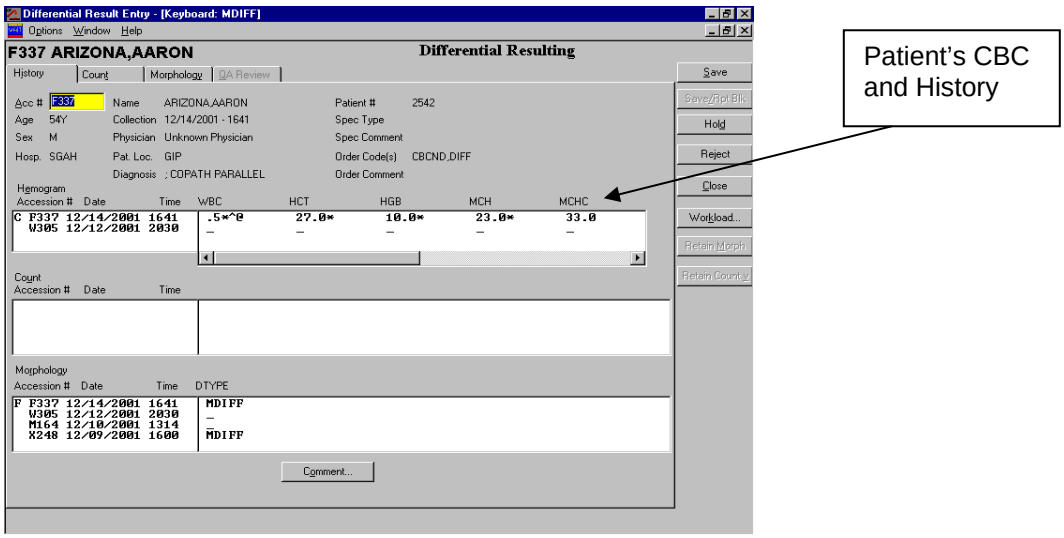
DIFF Keyboard: Accessing Differential Result Entry

1. Using GUI application, click on the **Differential Keyboard**. The following information dialog box displays.



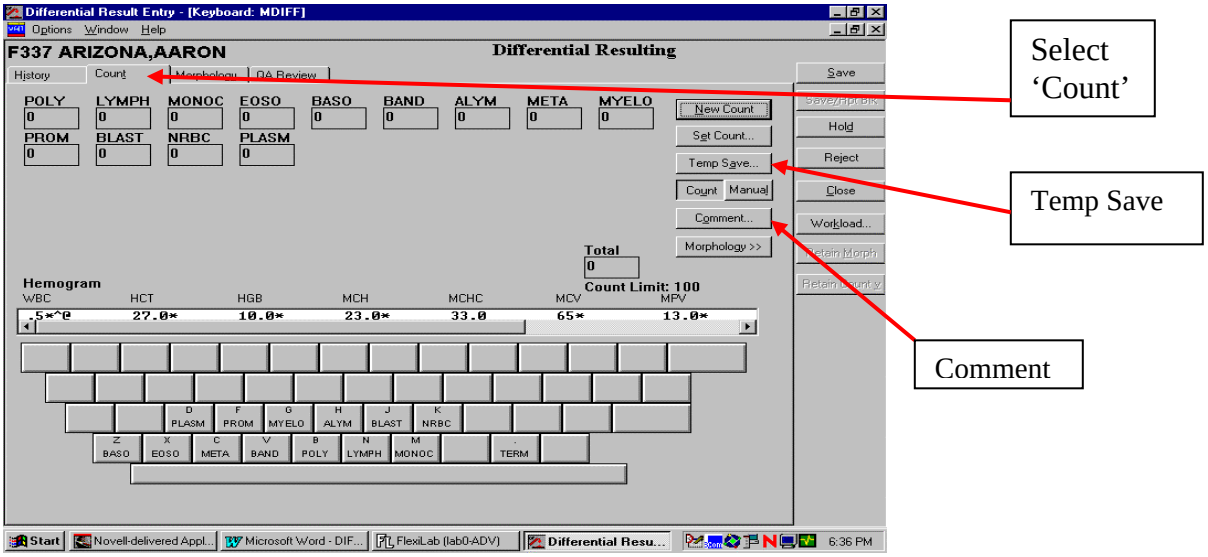
2. Click on **OK** to get to the next screen, differential resulting.

3. Type in the Accession # and press enter. The following keyboard will display:



4. To result a differential select **COUNT**. Then enter the differential using the keys on the keyboard or by using the mouse and clicking on the appropriate keys on the screen.

   a. If a second differential is required, save the first differential in the **Temp Save** file. After counting the second differential, also save in the Temp.Save file. Click on both counts, then click on **Average,** to get the average of both counts.
   b. If a comment needs to be appended to a cell, click on the appropriate cell then click on the comment box. Input the comment using free text in the Comments Box, or using text code in the Text Code Box.



   c. To delete a cell from the count, depress **Ctrl** from the keyboard and the count key for the cell that needs to be deleted.

5. To result morphology, select **MORPHOLOGY**. The following display will be seen:

6. Enter the morphology result using the keys on the keyboard or the mouse to click on the appropriate selection. Always result the **quantifier** before resulting the **qualifier,** i.e., 1+ ANISO

7. A quality assurance check must be performed before any result can be saved. Defaults are applied to cell counts and morphology results. To save and file all results, click on the **SAVE** button.

ADDENDUM 10

Differential Flagging Criteria

Criteria	Criteria Action Limit	Action 1	Action 2
Counts	Diff reflex criteria are applied once every 48 hours assuming no significant result changes		
WBC	≤2 or ≥30	Check for clots, repeat sample	SCAN
PLT	≤50	Check for clots, Vortex 1-2 min, repeat sample	SCAN
PLT	>50 ≤100, no flag	SCAN (first specimen)	
NE%/Poly	≥90, no flag or NE1	SCAN	
NE%/Poly	≥90, w/flag	DIFF	
LY%/Lymph	≥70	DIFF	
MO%/Mono	≥22	DIFF	
EO%/Eosin	≥25	SCAN	
BA%/Baso	≥3.0	Mix for 5 minutes, repeat	If unresolved, SCAN

- **DIFF all Patients ≤ 1yr; For patients <12 years old perform DIFF if the count is inverted**

Anemia	Morph reflex criteria are applied once every 48 hours assuming no significant result changes		
MCV	<70 or >110	MORPH	
RDW	>21	MORPH	
Microcytosis 1+	No		
Aniso 1+	No		
Aniso2+	No		
Aniso3+	Yes	MORPH	

- **All specimens meeting the SCAN and DIFF criteria must be evaluated for Pathology Review**

Flags			
NE1	No		
NE2	Yes	DIFF	
Blasts	Yes	DIFF	PATH Review
R or *	Yes	Vortex 1-2 min, Repeat	If unresolved, DIFF
Cell Inter	Yes	Vortex 1-2 min, Repeat	If unresolved, DIFF
Variant Ly	Yes	DIFF	
VERITY DIFF	Yes	DIFF	
Platelet Clump	Yes	Vortex 1 min, Repeat	If unresolved, PLT EST
Giant Platelts	Yes	Vortex 1 min, Repeat	If unresolved, PLT EST
NRBC	YES	SCAN, Remove if not seen on smear	