TRAINING UPDATE

Lab Location:	GEC	**Date Distributed:**	1/5/2015
Department:	Core	**Due Date:**	1/31/2015
		Implementation:	**2/1/2015**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Coulter AcT10 Operation for Complete Blood Count GEC.H11 v1

Description of change(s):

Most significant change is documenting QC in Unity instead of SQ

Section	Reason
3.2	Add tube sizes
5.3	Clarify process, add reference materials
6.3	Change documentation to action log
6.6	Replace LIS steps with Unity RealTime
8.1, 8.2	Add reference documents
10.3,11.2, Addenda 1	Replaced $10^3/\mu L$ units with x10(3)/mcL
10.5	Remove extraneous information
16	Add PM log
Addenda 3 & 6	Change reference to LH750 SOP
Addenda 8	Guide moved from related documents

This revised SOP will be implemented on February 1, 2015

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

Approved draft for training (version 1)

Technical SOP

Title	**Coulter AcT10 Operation for Complete Blood Count**
Prepared by	Cynthia Reidenauer Date: 5/15/2013
Owner	Robert SanLuis Date: 5/15/2013

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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1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Hemogram *(WBC, RBC, HGB, HCT, MCV, MCH, MCHC , PLT)*	Coulter Automated Hematology Analyzer, Ac●T10	CBCND
Platelet Count		PLTC

Note: The Ac●T10 does not perform a differential. During its use, all differentials must be performed manually.

Abbreviation	Term	Abbreviation	Term
WBC	White Blood Cell	MCHC	Mean Corpuscular Hemoglobin Concentration
RBC	Red Blood Cell		
HGB	Hemoglobin	PLT	Platelet
HCT	Hematocrit		
MCV	Mean Cell Volume		
MCH	Mean Corpuscular Hemoglobin		

Department
GEC – Germantown Emergency Center

2. ANALYTICAL PRINCIPLE

The Coulter Principle accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle in a conductive liquid passes through a small aperture using the Hematology analyzers. Hemoglobin, released by hemolysis, is converted to a stable cyanide containing pigment and measured by photometric absorbance.

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
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 = $\frac{\text{HGB x 100}}{\text{HCT}}$

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 2.7 mL Sodium Citrate – for platelet counts only		
Collection Container	4mL Lavender Top Tube or Microtainer 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	

Transport Container and Temperature	Same as above. Transport at room temperature or refrigerated.			
Stability & Storage Requirements	Room Temperature (18-25ºC): 24 hours Refrigerated: 24 hours. 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 13.7*
	Hemolysis	Slight pink OK	Pink OK	Cherry Red Unacceptable
	Lipemia	OK	OK	Milky = *see section 1378*
Other Interfering Specimens Factors	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 24 hours.			
Actions to Take for Rejected Specimens Message Codes & Notes	**Condition**	**Code**	**Comment**	
	QNS *(< minimum volume in 3.2)*	QNS	Quantity not sufficient to perform test. **Notify caregiver**.	
	Clotted	CLT	Specimen is clotted, unable to perform test. **Notify caregiver.**	
	Spurious results that will not duplicate	INT *or* UNSAT	Possible interfering substance. *or* Unsatisfactory specimen. **Notify caregiver.**	
	Gross hemolysis	HMT	Markedly hemolyzed. **Notify caregiver.**	
	Frozen or Past stability	UNSAT	Unsatisfactory specimen. **Notify caregiver.**	

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 / Kits	Supplier & Catalog Number
Ac●T Tainer	Beckman Coulter 8547111

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.

Assay Kit - Ac●T Tainer	
Reagent a	Coulter Balanced Electrolyte Solution - 4 liter
Reagent b	Coulter Lytic Reagent - 190 ml
Reagent c	Coulter Shutdown Diluent - 250 ml
Storage	2 - 25°C
Stability	Stable (when unopened) until expiration date on label. Opened stability: 60 days when operating environment is 16 - 25°C. 30 days when operating environment is 26 - 35°C.
Preparation	All reagents are received ready for use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

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

Caution: Controls contain 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 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.**

Calibrator	Coulter® S-Cal® Calibrator Kit
Preparation	Bring to room temperature prior to testing. Use within one hour. Check for visible signs of degradation prior to use, i.e. color change or clotting.
Storage/Stability	Store refrigerated (2-8°C). Opened expiration is 1 hour. Use within expiration date from manufacturer. For further details refer to the package insert.

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 electronics are installed. • When multiple levels of commercial controls are consistently out or biased for one or more parameters.
Procedure	Before Calibration: • Instrument has had PM in the last 6 months • Verify all routine maintenance is up-to-date. • Ensure maintenance for cleaning Baths has been performed. • Ensure you have sufficient supply of reagents to complete the calibration procedure. • Perform Shutdown. • Perform Startup. **Note**: The following steps require data to be manually calculated, as the Ac●T 10 does not calculate data for the calibration or precalibration steps. You may find the steps by referring to instructions in the Coulter Ac●T 10 Series Special Procedure and Troubleshooting manual. • Perform Reproducibility: 1. *If the CV% exceeds acceptability guidelines for any parameter listed in the Preventive Maintenance Procedures, this could be indicative of 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, this could be indicative of 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).*

Criteria	Special Notations	
	• All the above 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 in the Coulter Ac●T 10 Series Special Procedure and Troubleshooting manual.	
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.

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 *Retention of Records and Materials.*

6. QUALITY CONTROL

6.1 Controls Used

Control	Supplier & Catalog Number
Coulter 4C-ES Cell Control	Beckman Coulter ref #754188

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.

6.2 Control Preparation and Storage

NOTE: **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	Coulter 4C-ES Cell Control
Preparation	No control preparation is necessary. Follow instructions in the current control package insert for control handling. Bring to room temperature prior to testing.
Storage/Stability	• Store refrigerated at 2-8°C. • Observe expiration date. • Open vial stability: 35 days or 20 uses

6.3 Frequency

1. When the Ac●T10 is in use as a backup to the primary hematology instrument, all controls must be run every four hours at the designated time **+/- 30 minutes**. When the AcT10 is not in use as a backup, controls are run once per day after shutdown and startup.

Night Shift Daily QC

02:30 Run a 4C-ES Control on the Ac●T10 before shutting down the machine (rotate between the three 4C control levels; running a different level each day). Document the level used on the Ac●T10 Daily Maintenance Log.

02:40 Perform the Shutdown procedure. Let it stay in Shutdown for 30 minutes.

03:10 Perform the Start Up procedure, if all parameters PASS, run 3 levels of the 4C. File all start up print outs in the Ac●T10 Maintenance Logbook. Enter the QC into Sunquest and file all QC runs in the Ac●T10 Logbook. Document the daily maintenance in the Ac●T10 Maintenance Log.

2. **All three levels of 4C-ES control will be run when the Ac●T10 is placed into service as a backup, and every four hours afterward.**

   Document the time of the initial run in the LH750 Action Log.

3. **Once the primary instrument is back in service and the AC●T10 is no longer needed as a backup, the schedule of controls once per 24 hrs can be resumed.**

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 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 high	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

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
	Platelet Count	+/- 25%	5.0	8.7	8.5

QC Level	Parameter	Max. Total Allowable Error	Max CV, %	Max SD	SDc = Coulter Range / 3
Abnormal II low	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

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. Follow the steps / guidelines listed in the *Quality Control Program, Action for Unacceptable QC Results.*

Corrective Action

- Rejected runs must be effectively addressed by corrective action. Steps taken in response to QC failures must be documented in the 5C action log. 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 are printed and filed daily in the Ac●T10 Logbook.
- QC results are manually entered into Unity RealTime.
  The file is located in the "GEC EXPG1" folder
  ACAII: abnormal low is Level 1
  ACN: normal is Level 2
  ACA: abnormal high is Level 3
- The Ac●T10 does not retain any QC data; all QC is manually entered into the LIS from the printouts.
- QC records are printed monthly and maintained and available for a minimum of two *(2)* years.
- Patient results are reviewed and entered to the patient file via the LIS system. The Ac●T10 is not interfaced. Refer to section 10.5 for LIS resulting instructions.

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.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Brand	Instrument Model	Distributor
Coulter Electronics	Ac●T10	Beckman-Coulter, Inc. Technical Support 1 800 526 7694 Account number 942443

7.2 Equipment

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

7.3 Supplies

Other Items	Supplier and Catalog Number
Biohazard wipes	None specified
Applicator sticks	None specified

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. Refer to the Coulter Ac●T Series Special Procedures and Troubleshooting manual.

8.2 Start-up / Shut Down Procedures

Refer to the Ac●T10 Analyzer Reference, Operator's Guide (Addendum 8)

	Startup
1.	Initialize the Coulter. • Icon commands are always pictured in a box on the touch screen. • Coming out of Shutdown, touch the startup icon on the screen (up arrow). • Automatically occurs when powering up after turning the power off during a cycle or after a power interruption during a cycle. • Automatically occurs when powering on more than 2 hours after the previous sample was run. • Verify the Startup results are acceptable (PASS). If the results are acceptable, press the printer icon to the right of the PASS notations. If fail, press the Main screen icon and repeat Startup. May need to do 2-3 times. If fails after third time then call a Coulter Representative. • Return to Main screen icon.

	Shutdown
1.	Check Waste level.
2.	Touch the shutdown icon on the screen (down arrow).
3.	Clean work surfaces.

8.3 Test Sampling

Refer to the Ac●T10 Analyzer Reference, Operator's Guide (Addendum 8)

8.3	Running Whole Blood Samples
When you have set the Ac●T10 to the correct analyzing mode and have approved the sample ID, you are ready to run samples. IMPORTANT: Only run a whole-blood sample in the Whole Blood mode.	
1.	Set the analyzing mode to Whole Blood as indicated above.
2.	Set Sample ID to correct number.
3.	Present the mixed sample to the probe and press the aspirate switch. When you hear the beep, remove the samples. **Warning: The probe may contain biohazardous materials, including controls and calibrators. Keep hands away from the probe area. Probe moves up and down**
4.	The Ac●T10 displays the sample results on the screen.

8.3	Running Whole Blood Samples
5.	The results will print when the cycle is complete. Review the printouts for the following flags and refer to addendum 3 for action criteria • +++++ which indicates the parameter is over the reportable range • • • • • which indicates an incomplete result • - - - - - which indicates voteout or ***** for Aperture Alert (See the AC•T Special Procedures and Troubleshooting manual for further information. This is usually due to a possible clot that can be removed by Zapping the instrument three times.) **Code Type of Flag** * Review flag H High flag L Low flag + Exceeds linear range

8.4 Review of Patient Results

Step	Action
1.	Using function MEM 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
4.	Release all values that do not need to be repeated for delta values, critical values, or are not flagged on the Ac●T10 print out for review.
5.	Make a stained smear for the differential if there is one ordered.
6.	Follow the procedure for manual differential found in the HMX procedure GEC.H01.003

9. CALCULATIONS

MCV, MCH, MCHC are released from the Ac●T10 analyzer.

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 4 for calculation formulas.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

Any result rounded 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

10.4 Clinically Reportable Range (CRR)

Parameter	CRR Range
WBC	0.0 – 99.9 K/ µL
RBC	0.0 – 7.00 M/µL
Hemoglobin	0.0 – 25.0 g/dL
MCV	50.0 – 130.0
Platelet Count	0.0 – 999.0 K/ µL

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%

LIS Resulting for Patients
Function: **MEM**
Worksheet: **GHMAX**
Test: **CBCND**
Accept or Modify: M
WBC: HMXG
ACTG

Change the method code on each analyte to **ACTG** with the exception of RDW and MPV. Skip past those using the enter key.

Enter the Accession number and enter the patient results.
For RDW and MPV type **HIDE**.

Review all results for clerical errors before accepting them.

11. EXPECTED VALUES

11.1 Reference Ranges

See 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.
- **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.

13. PROCEDURE NOTES

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

13.1 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 - add CLMP to the report. NRBCs and/or megakaryocytes or giant platelets present - correct the WBC (refer to Addendum 6) No apparent cause - Have the test redrawn.

13.2 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.

13.3 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	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

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

13.4 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 result we provide 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*

13.5 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.

13.6 MCHCs greater than 36.5 or less than 29.0

If the MCHC is ≤ 29.0 or ≥ 36.5, it should be repeated one more time on the AcT●10 to rule out random error. If MCHC is still ≤ 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 still greater than 36.5, a slide should be made and examined 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, or the situation where the results are accurate due to the presence of spherocytes.

<table>
<tr><th>IF</th><th colspan="2">Then</th></tr>
<tr><td>Spherocytes are noted on the slide scan.</td><td colspan="2">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 colspan="2">Specimens with lyse resistant RBCs should be repeated. Dilute 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 5.</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 or icteria. 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 an Isoton 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:</td></tr>
</table>

IF	Then
	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 #4) 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, icteria or protein entities."*

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

Whenever the instrument gives a cellular interference flag, which on the AC●T10 is an asterisk, (*) a slide WBC estimate has to be done. If the estimate does not match within 20% of the WBC count a WBC correction has to be done. See section 13.2. Use the following calculation if this correction has to be done manually.

If slide review indicates presence of **>10nRBCs** or megakaryocytes, the WBC count must be corrected. 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.

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

13.8 Coulter Repeats

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

13.9 SCAN Smear

Refer to the HMX procedure GEC.H01.003 for scan instructions.

13.10 Correction of RBC & HGB in the presence of a WBC count greater than 150,000.

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 Ac●T10 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: $\text{MCHC} = \frac{\text{Corrected Hgb}}{\text{Corrected Hct}} \times 100$

13.11 Carryover in the presence of a WBC count greater than 25,000.

Carryover may occur when a sample with WBC > 25,000 is immediately followed by an abnormally low WBC count.

The following guidelines will be used:

IF	Then
WBC less that 1,500	Repeat the sample after itself and use the second run. This will eliminate carryover, no matter what the last specimen's value.
WBC greater than 25,000	Run a normal sample afterward to ensure the carryover will not affect the next sample.

13.12 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 Ac●T10 Series Diluent.
- For results greater than the CRR, report according to the chart.

Parameter	Dilutions for CRR	
	Ac●T10	Report as…
WBC	1:2	>150 x 10^3
RBC	1:2	>8.00 x 10^6
HGB	1:2 DO NOT DILUTE NEWBORNS	>25.0
PLT	1:2	>999.0

14. LIMITATIONS OF METHOD

14.1 CBC-Line Linearity

- Lin-C Linearity is an assayed material used to establish the Analytical Measurement Range and verify the calibration of the AcT10. 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 PN 7508033-A

14.2 Analytical Measurement Range (AMR)

Parameter	Analytical Measurement Range
	AC●T10
WBC	0 - 99.9
RBC	0 - 7.0
HGB	0 - 25.0
PLT	0 - 999.0
MCV	Linearity not evaluated

14.3 Imprecision

Imprecision is stated in terms of CV for the CBC parameters. Imprecision was determined by simple replicate testing (n-31) with normal whole blood, 4C PLUS cell control at three different levels and by difference analysis of paired tests with clinical specimens.

4C PLUS Normal Cell Control

Parameter	Mean	CV%
WBC	9.85	1.37
RBC	4.14	1.24
HGB	12.88	0.20
MCV	86.35	0.55
PLT	203.31	2.16

4C PLUS Abnormal Low Cell Control

Parameter	Mean	CV%
WBC	4.48	2.49
RBC	2.40	1.21
HGB	6.86	1.18
MCV	76.54	0.37
PLT	63.32	4.50

4C PLUS Abnormal High Cell Control

Parameter	Mean	CV%
WBC	19.44	0.96
RBC	5.19	1.02
HGB	17.66	0.80
MCV	93.81	0.24
PLT	377.41	2.81

Whole Blood in K3EDTA

Parameter	Mean	CV%
WBC	6.78	1.37
RBC	5.12	1.00

HGB	15.32	0.78
MCV	86.35	0.55
PLT	216.37	3.45

14.4 Interfering Substances

See 13.5

14.5 Clinical Sensitivity/Specificity/Predictive Values

Not applicable.

15. SAFETY

The employee has 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 Data Safety sheets
- Ac●T10 Reference Manual
- Critical Values (Lab policy)
- Delta Check policy
- Quality Control Program policy
- CUM or ICUM, LIS procedure
- Laboratory Safety Manual
- Tip Sheet for Operating Ac●T10 (AG.F254)
- Coulter AcT 10 Analyzer Maintenance (AG.F255)
- Current package inserts for Coulter® S-Cal® Calibrator Kit Package, Coulter® 4C-ES® Cell Control

17. REFERENCES

1. Coulter AcT Hematology Analyzer Reference Manual (PN 4237288 2003)
2. Coulter® S-Cal® Calibrator Kit Package insert, Beckman Coulter, 2009.
3. Gulati GL, Asselta A, Chen C. Using a vortex to disaggregate platelet clumps. Laboratory Medicine. 1997;28:665-667.
4. Rodak B, Fritsma G, & Doig, K. “Hematology: Clinical principles and Applications”, Third edition, 2007, pp 176-177.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
000	10/20/14	3.2	Add tube sizes	H. Genser	R SanLuis
000	10/20/14	5.3	Clarify process, add reference materials	H. Genser	R SanLuis
000	10/20/14	6.3	Change documentation to action log	H. Genser	R SanLuis
000	10/20/14	6.6	Replace LIS steps with Unity RealTime	H. Genser	R SanLuis
000	10/20/14	8.1, 8.2	Add reference documents	H. Genser	R SanLuis
000	10/20/14	10.3,11.2, Addenda 1	Replaced 10^3/µL units with x10(3)/mcL	L. Barrett	R SanLuis
000	10/20/14	10.5	Remove extraneous information	H. Genser	R SanLuis
000	10/20/14	16	Add PM log	L. Barrett	R SanLuis
000	10/20/14	Addenda 3 & 6	Change reference to LH750 SOP	L. Barrett	R SanLuis
000	10/20/14	Addenda 8	Guide moved from related documents	L. Barrett	R SanLuis
000	10/20/14	Footer	version # leading zero’s dropped due to new EDCS in use as of 10/7/13	L. Barrett	R SanLuis

19. ADDENDA

Addendum	Title
1	Reference Ranges
2	CBC Diff/Scan Action and Repeat Criteria
3	Decision Rules, Flags and Action Criteria
4	Calculation Formulas
5	Plasma Replacement
6	Smear Review and Manual Differential
7	Differential Flagging Criteria
8	Ac●T10 Series Analyzer Operators Guide

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

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

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

ADDENDUM 2

REPEAT CRITERIA

KEY	
RPT –	Repeat CBC on AcT
SCAN –	Microscopically scan smear & perform manual differential if required (refer to Addendum 6)

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).*
	≥99.9	RPT by Dilution	• Re-analyze by dilution. Refer to AMR limits. Add comment RESULTS VERIFIED BY REPEAT ANALYSIS. • Refer to WBC ≥30.0
	WBC * flag	SCAN	• Ensure 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.

Parameter	Condition		Action Needed
HGB (con't)	≥ 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
			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	Not applicable		Note: The AC●T10 does not perform an RDW. This parameter will have to be answered with the word HIDE.
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 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, platelet clumps, platelet satellitism, fibrin, NRBCs, RBC fragments, or old

Parameter	Condition		Action Needed
Platelet (cont)			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*
	≥ 999	RPT by dilution, SCAN	• Re-analyze by manual dilution with Coulter Diluent. Correlate with morphology review. Add comment REP = *RESULTS CONFIRMED, TEST REPEATED*

ADDENDUM 3

Decision Rules, Flags and Action Criteria for the AC●T10

Condition (IF)	Action Needed (THEN)
WBC *	Check for clots, vortex,repeat . If not resolved perform a WBC and PLT estimate before reporting
WBC---- or RBC--- or HGB--- or PLT---	Check for clots and Rerun Sample / Troubleshoot
WBC +++ or RBC+++ or HGB+++ or PLT+++	Dilute Sample, Repeat, Run AcT10 diluent Blank
PLT *	Vortex 1-2 minutes, Repeat, If unresolved remove PLT result before releasing the Hemogram Review Smear for PLT estimate Refer to Section 13.6
H&H Check Failed	Mix sample for 5-10 minutes then repeat. Verify Indices correspond, scan diff if necessary

Note: Refer to Addendum 8 in LH750 procedure GEC.H217 for the Manual Differential

ADDENDUM 4

Calculation Formulas

MCV = (Hct x 10) / RBC

MCH = (Hgb / RBC) x 10

MCHC = (Hgb / Hct) x 100

ADDENDUM 5

Plasma Replacement with Warm Ac●T10 Diluent

Dispense 5 ml of AcT10 Diluent into a plastic tube with a tight fitting lid. Place the tube, sealed with parafilm, in the 37°C heat block for a minimum of 15 minutes.

Meanwhile, spin a 2 mL aliquot of the patient's 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 Ac●T10 Diluent, mix thoroughly and run IMMEDIATELY through the Ac●T10.

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 6

SMEAR REVIEW AND MANUAL DIFFERENTIAL

The AC●T10 does not report differentials. Refer to the LH750 procedure, GEC.H217 regarding smear review, staining and differential reporting.

ADDENDUM 7

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)	
Anemia	Morph reflex criteria are applied once every 48 hours assuming no significant result changes		
MCV	<70 or >110	MORPH	
• **All specimens meeting the SCAN and DIFF criteria must be evaluated for Pathology Review**			
Flags			
R or *	Yes	Vortex 1-2 min, Repeat	If unresolved, DIFF

Addendum 8

Ac•T10 Series Analyzer Operators Guide

Using This Document

Use the A^{c}•T Series Analyzer Operator's Guide as a quick reference tool. Before attempting to use this card to operate the A^{c}•T analyzer, read the A^{c}•T Series Analyzer Getting Started manual to become familiar with the instrument and its operating procedures. This card is **not** meant to teach you how to run the instrument.

Screen Icons

	Whole Blood Mode		Diluter Functions
	Predilute Mode		Continue
	A^{c}•T Tron‡ Mode		Print
	Analyzing Mode		Exit
	In Progress		Enter Patient ID
	Main Screen		Save and Exit
	Startup		Dispense
	Shutdown		Delete
	Sample Results		Darken Screen
	Diagnostics		Lighten Screen
	Setup		

Warnings/Instructions

To avoid being exposed to biohazardous material, adhere to standard laboratory safety procedures.

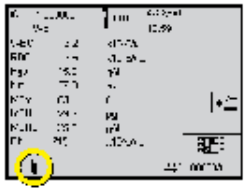
To avoid interference with probe movement, keep your hands away from the area after removing the tube.

Go to

‡ At the time of instrument release, this product was not available.

Run Samples - Whole Blood

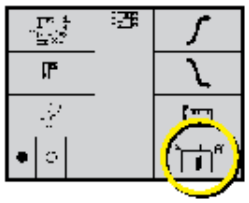
1



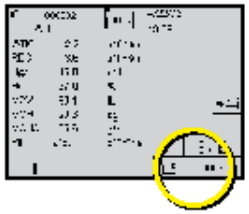
3

or

2



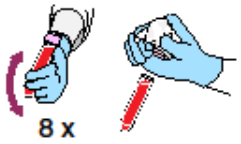
3



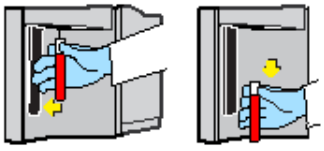
ID OK 4

or

4



8 x



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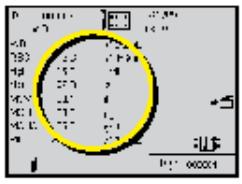


OK

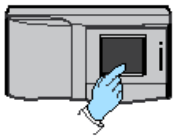
or

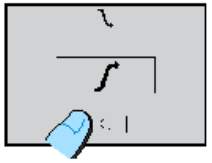
See review results

■ END

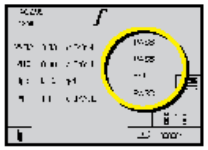
Startup (Power already on)

1





2

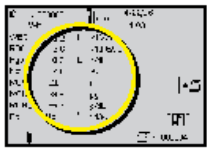


PASS
PASS
PASS
PASS

FAIL

■ END

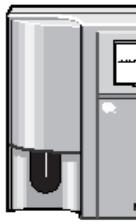
Review Results



H = High patient limit
L = Low patient limit
* = Review Results
----- = 2 of 3 count periods voteout
+++++ = Parameter out of operating range
***** = Aperture alert
..... = Incomplete Computation
+ = Parameter out of linear range

For more information, see the What Flags Mean Heading in the Special Procedures and Troubleshooting manual.

COULTER®
Series Analy

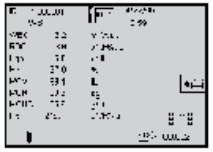


Run Controls

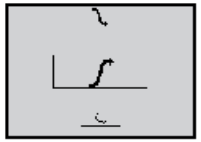
See package inserts of COULTER Control.

Shutdown

1



2



■ END

EC REP
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Mervue Business Park
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CE

PN 4237287BA August 2010

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