

Laboratory Procedure Manual

Analyte: **Complete Blood Count**

Matrix: **Whole Blood**

Method: **Complete Blood Count with 5-Part
Differential**

Method No.:

Revised:

as performed by:

Contact:

Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for NHANES 2003–2004 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
I25_c	LBXWBCSI	White blood cell count (SI)
	LBXLYPCT	Lymphocyte (%)
	LBXMOPCT	Monocyte (%)
	LBXNEPCT	Segmented neutrophils (%)
	LBXEOPCT	Eosinophils (%)
	LBXBAPCT	Basophils (%)
	LBDLYMNO	Lymphocyte number
	LBDMONO	Monocyte number
	LBDNENO	Segmented neutrophils number
	LBDEONO	Eosinophils number
	LBDBANO	Basophils number
	LBXRBCSI	Red cell count SI
	LBXHGB	Hemoglobin (g/dL)
	LBXHCT	Hematocrit (%)
	LBXMCVSI	Mean cell volume (fL)
	LBXMCHSI	Mean cell hemoglobin (pg)
	LBXMC	MCHC (g/dL)
	LBXRDW	Red cell distribution width (%)
	LBXPLTSI	Platelet count (%) SI
	LBXMPSI	Mean platelet volume (fL)

1. Clinical Relevance and Summary of Test Principle

Perform a complete blood count (CBC) in duplicate on all Sample Persons (SPs) age 1 and older. Perform the CBC on the Beckman Coulter MAXM. Run a CBC on home exam SP's EDTA blood tubes after returning to the MEC.

A. Purpose and Principle of Test

The Beckman Coulter method of sizing and counting particles uses measurable changes in electrical resistance produced by nonconductive particles suspended in an electrolyte.

A suspension of blood cells passes through a small orifice simultaneously with an electric current. A small opening (aperture) between electrodes is the sensing zone through which suspended particles pass. In the sensing zone, each particle displaces its volume of electrolyte. Beckman Coulter measures the displaced volume as a voltage pulse, the height of each pulse being proportional to the volume of the particle.

The quantity of suspension drawn through the aperture is for an exact reproducible volume. Beckman Coulter counts and sizes individual particles at a rate of several thousand per second. This method is independent of particle shape, color, and density.

The MAXM is a quantitative, automated, differential cell counter for in vitro diagnostic use.

The MAXM measures these parameters in whole blood:

Cell	Parameter	Measured	Pulse size wavelength calculation	Reported units
WBC	white blood cell count	WBC bath	≥ 35 fL	$n \times 10^3$ cells/ μ L
RBC	red blood cell count	RBC bath	36–360 fL	$n \times 10^6$ cells/ μ L
Hgb	hemoglobin concentration	WBC bath	525 nm	g/dL
Hct	hematocrit	computed	RBC \times MCV/10	%
MCV	mean cell volume	derived from RBC histogram	# \times size of RBC/total RBC	fL
MCH	mean cell hemoglobin	computer	Hgb/RBC \times 10	pg
MCHC	mean cell hemoglobin concentration	computed	Hgb/Hct \times 100	g/dL
RDW	red cell distribution width	derived from RBC histogram	CV expressed in % of the RBC size distribution	%
Plt	platelet count	RBC bath	2 to 20 fL	$n \times 10^3$ cells/ μ L
MPV	mean platelet volume	derived from Plt histogram	Mean volume of Plt population under the fitted curve \times constant	fL
NE%	neutrophil percent	derived from scatterplot	# cells inside NE area/# cells inside total cell area \times 100	%
NE #	neutrophil number	absolute number	NE%/100 \times WBC count	10^3 cells/ μ L

LY%	lymphocyte percent	derived from scatterplot	# cells inside LY area/# cells inside total cell area × 100	%
LY#	lymphocyte number	absolute number	Ly%/100 × WBC count	10 ³ cells/μL
MO%	monocyte percent	derived from scatterplot	# cells inside MO area/# cells inside total cell area × 100	%
MO#	monocyte number	absolute number	MO%/100 × WBC count	10 ³ cells/μL
EO%	eosinophil percent	derived from scatterplot	# cells inside EO area/# cells inside total cell area × 100	%
EO#	eosinophil number	absolute number	EO%/100 × WBC count	10 ³ cells/μL
BA%	basophil percent	derived from scatterplot	# cells inside BA area/# cells inside total cell area × 100	%
BA#	basophil number	absolute number	BA%/100 × WBC count	10 ³ cells/μL

*PDW – platelet distribution width and percent – platelet crit are NOT for diagnostic use and do not print. Coulter uses the value for PDW as an internal check on the reported platelet parameters, Pct and MPV.

Methodology: The methods used to derive CBC parameters are based on the Beckman Coulter method of counting and sizing, in combination with an automatic diluting and mixing device for sample processing, and a single beam photometer for hemoglobinometry. The WBC differential uses VCS technology. Analysis and classification of WBCs use three simultaneous measurements of individual cell volume (V), high frequency conductivity (C), and laser light scatter (S). The scattergram plots the cells based upon the measurements of these three parameters.

2. Special Safety Precautions

All specimens may be potentially positive for infectious agents including HIV and the Hepatitis B and C virus. Observe universal precautions. It is mandatory to wear gloves and lab coat when handling all human blood products and Beckman Coulter controls. Wear safety glasses whenever operating the instrument in SECONDARY mode. Dispose of all biological samples in a biohazard container and wipe down all work surfaces with 1% bleach solution at the end of each session.

The Mobile Examination Center (MEC) Hazardous Chemicals Manual contains all Coulter Material Safety Data Sheets (MSDS).

3. Computerization: Integrated Survey and Information System (ISIS)

The MAXM Data Management System (DMS) transmits individual SP results to the MEC automated ISIS system. Review all SP results at the Coulter DMS monitor. The hematology module in the laboratory application automatically receives the results or transmits them manually to the hematology module. The laboratory application evaluates the data for completeness and accuracy. The final decision to accept or reject a result is the responsibility of the medical technologist.

All data are backed up and stored at Westat's home office.

4. Procedures for Collecting, Storing, and Handling Specimens; Criteria for Specimen Rejection

A. Specimen collection

The phlebotomist collects a 3- or 5-mL K3 EDTA tube on all SPs aged 1 year and older following established venipuncture protocol and procedures (a 1-2% dilution effect occurs in this liquid EDTA tube).

B. Specimen preparation

Run the CBC as soon as possible; there is no requirement to wait any length of time between drawing the blood and running the CBC.

5. Procedures for Microscopic Examination

Not Applicable - Do not prepare differential microscopic slides.

6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation

The MAXM DMS stores and maintains the lot numbers and expiration dates.

A. Reagents, controls and calibrators

- (1) Isoton III -- PN 8546733 (20 L) -- an isotonic electrolyte
 - Expires on expiration date printed on container.
- (2) Beckman Coulter Clenz -- PN 8546930 (5L) -- a cleaning agent that cleans and rinses diluter parts
 - Expires 90 days after being opened and installed on the instrument.
- (3) Lyse-S III diff lytic reagent- PN 8546983 (1L) -- a lytic reagent used for the CBC mode
 - Expires 60 days after being opened and installed on the instrument.
- (4) Scatter Pak -- PN 8546917
 - Diff Lytic Reagent -- Erythrolyse II erythrocyte lytic reagent
 - Diff Leukocyte Preservative -- StabiLyse leukocyte preservative
 - Expires 60 days after being opened and installed on the instrument.
- (5) Latron Controls - PN 7546914 (5 x 16-mL)
 - Expires 30 days after being opened.
- (6) Latron Primer - PN 7546915 (5 x 16-mL)
 - Expires 30 days after being opened.
- (7) 5C Cell Controls Tri Pack contains Normal, Abnormal I, Abnormal II - PN 7547001 (9 x 3.3-mL)
 - Expires 13 days or 13 events after being opened.

- (8) Calibration S-CAL - PN 7546808 (2 x 6-mL) (With Lin-CPN 6605374)
 - Use at the start of each stand. Expires 1 hour after being opened.
- (9) 5C Cell control normal - PN 7546923 (9 x 3.3-mL)
 - Use when calibrating with S-CAL for reproducibility study.

B. Supplies

- (1) 3-mL K3 EDTA Becton Dickinson Hemogard Vacutainer tube
- (2) Tube rocker
- (3) Clorox Bleach, 5.25% sodium hypochlorite 1-800-292-2200
- (4) Bottled distilled water
- (5) Three 30 mL plastic containers with lid
- (6) Two one-liter containers with lid
- (7) Plastic squirt bottle
- (8) Cotton gauze pads
- (9) Three-hole paper punch
- (10) Notebook
- (11) Fujitsu printer ribbon
- (12) Flashlight
- (13) 10-mL syringe with plastic tubing
- (14) Precision screwdriver set
- (15) Distilled water bottle
- (16) Disposable lab jacket, 48 inches long

C. Notes

- (1) If reagents become frozen in transit, mix thoroughly by inversion and let bubbles settle before use.
- (2) Do not place reagents on or near electric cords or lines to avoid electrical interference.

7. Calibration and Calibration-Verification Procedures

S-CAL (PN 7546808 - 2 x 6-mL) The S-CAL calibrator kit determines the adjustment factors for the calibration of the Beckman Coulter MAXM. Calibration is a procedure to standardize the instrument by determining its deviation from calibration references and to apply any necessary correction factors. Perform calibration in the close-vial mode, at ambient room temperature range (16-32°C, 60-90°F), using S-CAL as an alternative to whole blood.

A. Perform calibration:

- At the beginning of each stand
- After replacing any component dealing with dilution preparation, such as BSV or primary measurement, such as an aperture
- If the Beckman Coulter representative suggests calibration
- If controls show unusual trends or are outside limits

- When room temperature varies more than 10°F (5.5°C) from the room temperature during the last calibration

- (1) Principle - The MAXM uses the S-CAL kit that requires a calibrator to convert electronic measurements of each sample into accurate results expressed in clinical terms. S-CAL calibrates the WBC, RBC, Hgb, MCV, Plt, and MPV parameters. It is a stabilized human-blood preparation. S-CAL is an acceptable alternative to whole blood calibration.

The calibration procedure uses replicate measurements of S-CAL calibrator. The S-CAL divides the average result into the calibrator Assigned Value to give the Adjustment Factor. Then, it obtains and adjusts an instrument reading according to the Adjustment Factor.

Hct, MCH, MCHC, RDW, and the DIFF parameters do not require calibration.

Reagents - S-CAL consists of treated, stabilized, human erythrocytes and platelet sized components in an isotonic bacteriostatic medium. Fixed erythrocytes simulate leukocytes.

Materials required -- Before calibrating, assemble the following materials:

S-CAL kit containing two 6-mL vials of S-CAL calibrator, one plastic pipette, and two empty glass-mixing tubes with pierceable caps.

- (2) Storage, handling and stability -- Sealed vials are stable through the expiration date when stored at 2–8°C (35–46°F). Open and/or pooled vials are stable for 1 hour.

Potential biohazard – Each human donor used in preparation of this material was tested by an FDA-approved method for the presence of the antibodies to Human Immunodeficiency Virus (HIV) and Hepatitis C (HCV), as well as for Hepatitis B surface antigen, and found to be negative (were not repeatedly reactive). Handle these reagents at Biosafety Level 2 because no test method can offer complete assurance that these and other infectious agents are absent.

Indications of instability or deterioration -- The supernatant should show no gross turbidity and be light red in color. Significant darkening of the supernatant might indicate hemolysis and a reduction of the red cell count.

B. Pre-calibration, reproducibility, and carryover check

Perform a calibration after the instrument has been "Shut Down" for at least 30 minutes. Select DILUTER FUNCTIONS, START-UP.

- (1) Pre-calibration procedure

Bleach the aspiration system using the Clean Needle procedure.

- (a) Prepare a fresh bleach solution. Put one-mL Clorox bleach and 1 mL of distilled water into 5-mL lavender top Vacutainertube.
- (b) Stop the system before it performs the Clean Needle procedure. If status message is not Select Function or Compressor Off, go to SAMPLE ANALYSIS, RUN SAMPLE, [F3], Run. Press [F9] Stop.
- (c) Select SPECIAL FUNCTIONS, DIAGNOSTICS, OPERATOR OPTIONS, FLUIDIC TESTS, and CLEAN NEEDLE. Press [Enter] and follow the screen instructions. Wait for green light before placing tube into the carousel.

To rinse the system, perform daily Start Up. Select DILUTOR FUNCTIONS; START UP [Enter].

Run 5C cell controls.

- (2) Reproducibility check

- (a) Select SAMPLE ANALYSIS, RUN SAMPLES. Press [Enter]. Select [F5] to access options. Change [F5] Print: to none and [F6] Host to Off.
- (b) Use an unopened, normal-level, 5C cell control for reproducibility studies. Label the control vial with date and initial. Use of expired controls for this procedure is acceptable.
- (c) Select SPECIAL FUNCTIONS, CALBRATION, REPRO-DUCIBILITY [Enter].
- (d) Is "Sample Mode?" displayed? If YES, go to step 3. If NO, press [F9] Stop.

- (e) Press [F6]. Press space bar to turn the DIFF ON. Press [Enter]. Press [F2] START PRIMARY.
- (f) Does the following message appear? "MODE REQUIRES EXISTING RUNS TO BE DELETED. ARE YOU SURE?: NO." If the message appears, press the space bar to answer "yes." Press [Enter]. This deletes the old data.

If there are runs present in the table and the message does not appear, press [F8] Delete. Press the space bar to answer "YES." Press [Enter]. This deletes the old data.

- (i) Follow the directions listed in Section X for preparation and mixing the 5C-cell control.
- (ii) Cycle the sample 11 times. Do not use the bar code reader. Mix gently before each cycle. Insert the tube into the carousel when the green light appears. The first sample is automatically marked DEL and its results are not included in the calculations.
- (iii) Check the results. Verify the average %CV does not exceed these limits:

- WBC 2.5%
- RBC 2.0%
- Hgb 1.5%
- MCV 2.0%
- Plt 5.0%
- MPV 3.0%

Check the low to high difference (bottom line, right hand side) for the diff parameters with these limits:

- LY% ≤ 4.8
- MO% ≤ 3.2
- NE% ≤ 4.8
- EO% ≤ 1.6
- BA% ≤ 1.6

If the results are outside the limits, call the Beckman Coulter representative.

- (iv) Press [F4] to print the screen for the logbook. Press [F3] Run [F9] STOP. Press [Esc]. Proceed with a Carryover Check.
- (g) Notes
- (i) Be sure the PR↓ is on the status line before starting the reproducibility study.
 - (ii) Be sure to delete any data on the reproducibility screen before starting a new study.
 - (iii) On the reproducibility screen, use the %CV line for CBC parameters only and the Diff line for the DIFF parameters only.
- (3) Carryover check (Draw two 5-mL EDTA tubes of normal blood)
- (a) Select SPECIAL FUNCTIONS, CALBRATION, and CARRYOVER. Press [F2] START PRIMARY. Does the following message appear? "MODE REQUIRES EXISTING RUNS TO BE DELETED. ARE YOU SURE?: NO." If the message appears, press the space bar to answer "yes." Press [Enter]. This deletes the old data.
 - (i) Use a routine 5-mL Vacutainer EDTA (BD #369614) for the carryover check clean vials. Fill three separate vials with diluent, then cap.
 - (ii) Follow the screen directions. Cycle two vials of normal blood and three vials of diluent. Do not use the bar code reader. Insert the tube into the carousel when the green light appears.

- (iii) Check the lower right corner of the screen for CARRYOVER ACCEPTABLE. If the result is not acceptable and the H flags appear next to the carryover value results, repeat the procedure. If results are still OUT, call the Beckman Coulter representative.
- (iv) Press [F4] to print the carryover screen for the logbook. Press [F9] to return to the main menu.

(b) Notes

- (i) Choose a sample with a WBC count of $10,000 \pm 1,000$ if possible. All other parameters should be within normal ranges.
- (ii) Ignore count period (CP) voteouts on diluent runs.
- (iii) Percent carryover is calculated by the formula.
- (iv) $1\text{st diluent sample} - 3\text{rd diluent sample} \times 100 = \% \text{ carryover } 2\text{nd sample}$.

C. CBC calibration with S-CAL calibrator

Take the S-CAL calibrator from the refrigerator. Let it warm to ambient temperature for 20 minutes. Meanwhile, continue with the following steps:

- (1) Select SPECIAL FUNCTIONS, CALBRATION, and CBC CALBRATION. Press [F2] START PRIMARY. Delete any data in the table. Press [F8]. Press the space bar to answer "yes." Press [Enter].
- (2) Prepare the CBC calibration screen.
 - (a) Enter the S-CAL calibrator assigned values from the Table of Assigned Values on the package insert on the REF: VALUES line at the cursor. The Hct is not calibrated although the value is listed on the package insert.
 - (b) Enter the expiration date and the lot number.
- (3) Prepare and cycle the calibrator
 - (a) Mix by hand as follows: Roll the tube slowly between the palms of the hands eight times in an upright position. Invert the tube and slowly roll it again eight times. Gently invert the tube eight times. Repeat these actions again.
 - (b) Determine that all cells are uniformly distributed by inspecting the vial contents. If the cells are not totally distributed, repeat the mixing procedure. Use cotton gauze pads to remove the rubber stopper from the vial. Absorb any spray produced by pressure within the vial by using the gauze pads, avoiding accidental contact with the product.
 - (c) With the pipette, fill each of two mixing tubes to within 1/4 inch of the top. Dispose of extra S-Cal. Set one of these tubes aside in a test tube rack. Do not refrigerate or place on rocker. Use Tube #1 for calibration and tube #2 is for verification if needed.
 - (d) Cycle the first cap-pierceable calibrator 11 times. Do not use the bar code reader. Invert the vial and mix the S-CAL calibrator before each cycle. Insert the tube into the carousel when the green light appears. The first sample is automatically marked DEL and its results are not included in the calculations.
 - (e) After the cycles are complete, press [F4] Print to print the screen for the logbook. Press [F3] Run, [F9] STOP. Press [Esc.]
- (4) Assess the run
 - (a) Check for trending. If trending is present, stop and contact a Beckman Coulter representative.
 - (b) Check the %CV values against these precision %CV limits. If any parameter exceeds the limit, STOP and contact a Beckman Coulter representative.

Parameter	Precision (CV%)
WBC	≤2.5
RBC	≤2.0
Hgb	≤1.5
MCV	≤2.0
Plt	≤5.0
MPV	≤3.0

- (c) FAC %DIFF values against these limits (disregard minus signs. The values are absolute numbers). If any parameter exceeds its limit, STOP and call a Beckman Coulter representative.

Parameter	Acceptable FAC %DIFF
WBC	≤5.0
RBC	≤2.0
Hgb	≤3.0
MCV	≤2.5
Plt	≤9.0
MPV	≤20.0

- (d) Determine the need for calibration. Check the FAC %DIFF and DELTA DIFF values against these limits (disregard minus signs. The values are absolute numbers).

Parameter	Do not calibrate if FAC %DIFF is:	Do not calibrate if DELTA DIFF is:
WBC	≤1.25	≤0.10
RBC	≤0.70	≤0.03
Hgb	≤0.78	≤0.10
MCV	≤1.18	≤1.00
Plt	≤2.70	≤6.0
MPV	≤5.00	≤0.50

- (e) If both the FAC %DIFF and DELTA DIFF values for all parameters are less than or equal to these limits, DO NOT calibrate: Press [F4] to print the screen for the logbook.
- (f) If the FAC %DIFF or DELTA DIFF value of any of these parameters is greater than these limits continue with the next steps to verify the calibration.
- (5) To verify calibration:
- (a) Determine which calibration factors to change. Check the FAC % DIFF and DELTA DIFF values against these ranges.

Parameter	Calibrate if FAC %DIFF is:	Calibrate if DELTA DIFF is:
WBC	>1.25 but ≤5.00	>0.10 but ≤0.40
RBC	>0.70 but ≤2.00	>0.03 but ≤0.09

Hgb	>0.78 but ≤3.00	>0.10 but ≤0.40
MCV	>1.18 but ≤2.50	>1.00 but ≤2.00
Plt	>2.70 but ≤9.00	>6.0 but ≤20.0
MPV	>5.00 but ≤20.00	>0.50 but ≤2.00

- (b) If any value is outside these ranges, then, is Select Function displayed? If NO, press [F3] Run [F9] STOP [Esc]. If YES, Press [F5]. Choose SELECT PARAMETERS. Press [Enter]. Use the space bar to select YES for parameters that need adjusting, NO for others. Press [Esc]. Select TRANSMIT FACTORS. Press [Enter]. The following message appears: "WARNING -- DATA WILL BE CLEARED AFTER TRANSMISSION. DO YOU WANT TO PRINT DATA? Y/N" Select N since the values have already been printed.
- (c) Press [F3] Run [F2] START PRIMARY. Gently invert the second cap-pierceable vial of S-CAL calibrator 8-10 times. Cycle the S-CAL calibrator 11 times. Before each cycle, invert the vial and mix the S-CAL calibrator. Check the FAC %DIFF and DELTA DIFF values results for all parameters against these limits (disregard minus signs) to be sure that they are below the lower limit.

Parameter	FAC %DIFF	DELTA DIFF
WBC	≤1.25	≤0.10
RBC	≤0.70	≤0.03
Hgb	≤0.78	≤0.10
MCV	≤1.18	≤1.00
Plt	≤2.70	≤6.0
MPV	≤5.00	≤0.50

- (d) If any value is above these limits, call a Beckman Coulter representative. Turn the printer back on using Options. Press [F4] to print the screen for the logbook. Rerun controls.
- (6) Calibration terms and formulas
- (a) MEAN
- The average of the 10 S-CAL calibrator runs
- (b) NEW CALFAC
- The calibration factor needed to obtain the S-CAL Calibrator Reference value. The instrument calculates and displays it whether or not it needs to be changed.
 - Reference Value x Old Cal Factor = NEW CALFAC
 - S-CAL mean value (n=10)
- (c) OLD CALFAC
- The current calibration factor
- (d) %CV
- Indicates the reproducibility of the S-CAL calibrator run. Check to ensure that the data being used is valid when making decisions to recalibrate or not.
- (e) FAC % DIFF
- The difference between the OLD CALFAC and the NEW CALFAC expressed as a percentage.
 - NEW CALFAC - OLD CALFAC × 100 = FAC %DIFF
 - OLD CALFAC

- (f) DELTA DIFF
 - The absolute difference between the reference value and the S-CAL calibrator MEAN.
- (g) REF VALUES.
 - The assigned value for each parameter.

8. Assay Procedure

- (1) Run Start Up at the beginning of the first session each operational day.
- (2) Leave the instrument on and operate the MAXM with all panels closed. Check to make sure the date and time displayed are correct. To set date and time select SPECIAL FUNCTIONS, SET UP, SYSTEM SET UP, SET DATE/TIME. Move the cursor and enter the correct date or time and press [Enter] or the arrow keys. Press [F10] to save and exit.
- (3) To begin Start Up, select DILUTER FUNCTIONS, and START UP. Press [Enter]. Follow the instructions on the screen.
- (4) After Start Up is complete, evaluate the display. Expired reagents and failed checks appear in red. Press [F2] to view detailed results. Make sure the Background and other Start Up results are within limits. Results outside limits turn red. Results automatically print.
- (5) Review and verify sample analysis set up.
- (6) Select SAMPLE ANALYSIS, RUN SAMPLE [F3]. Sample mode? Press [F5] for options. Press the corresponding function key to enter specific options. Use [F6] to turn the Diff ON. Use [F5] to change XB and DB to ON, Print to NONE, Host to ON, and Display Only to OFF. Press [Esc] to exit.
- (7) Run controls
- (8) Run Beckman Coulter LATRON primer and latex control, and all three levels, normal (blue), abnormal I (yellow), and abnormal II (pink), of Beckman Coulter 5Ccell control daily at the beginning of the first session. Run all three levels (normal, abnormal I, and abnormal II) of Beckman Coulter 5Ccell control at the beginning of the second session.
- (9) Run Sample Persons
- (10) Review the placement of the bar code on the sample tube to make sure it is parallel to the stopper and does not cover the bottom of the tube.
- (11) Run all samples in duplicate.
- (12) Run samples in the primary mode.
- (13) Check to verify connection and working order of the printer.
- (14) Select SAMPLE ANALYSIS, RUN SAMPLES. Press [Enter].
- (15) Is "Sample Mode?" displayed? If no, press [F9] STOP. Use [F6] to turn Diff ON.
- (16) Press [F2] START PRIMARY.
- (17) Identify the sample by holding the bar code label on the tube in front of the reader. Green light will beep -- Place the tube in the carousel. If the red light appears, wait and try reading the bar code label again.
- (18) Alternatively, cycle samples in the SECONDARY mode.
- (19) Blood detectors are inactive in the SECONDARY mode. This mode does not check sample and aspiration integrity. Run samples in the SECONDARY mode only if the primary mode is unavailable.
- (20) Select SAMPLE ANALYSIS, RUN SAMPLES. Press [Enter].
- (21) Is "Sample Mode?" displayed? If no, press [F9] STOP. Use [F6] to turn Diff ON.
- (22) Press [F3] SECONDARY.
- (23) Identify the sample by holding the bar code label on the tube in front of the reader. Green light will beep. If the red light appears, wait and try reading the bar code label again.

- (24) Cycle the sample in the SECONDARY mode:
- (25) Mix the sample gently.
- (26) Open the tube and immerse the aspirator tip in the sample.
- (27) Press and release the sample bar.
- (28) Remove the sample when the instrument beeps.
- (29) Monitor the aperture-viewing screens for debris during the COUNT period.
- (30) Daily Shut Down
 - (i) Shut down the instrument at the end of each day. Shut down the instrument for at least 30 minutes but less than 48 hours.(i)
 - (ii) To begin shut down make sure the status line displays Select Function. Select DILUTER FUNCTIONS and SHUT DOWN. Press [Enter]. (ii)
- (31) When it is time to use the instrument, perform Start Up. Results must be within tolerance.
- (32) Perform daily QC and operate as usual.
- (33) Prolonged Shut Down
- (34) If the instrument is going to be shut down for 48-72 hours, perform the following steps:
- (35) Perform daily Shut Down. Let cleaning agent remain in the instrument for at least 30 minutes.
- (36) Perform Start Up to put diluent in the system.
- (37) Turn off the power.
- (38) When it is time to use the instrument, turn power on, and perform a Start Up. Results must be within tolerance.
- (39) Perform daily QC and operate as usual.
- (40) If the instrument is going to be shut down for more than 72 hours, perform the following steps:
- (41) Place all reagent pickup tubes into distilled water.
- (42) Repeatedly perform Shut Down and Start Up procedures throughout the day.
- (43) Let distilled water remain in the reagent lines.
- (44) Turn off the power.
- (45) Place reagent caps on all open containers.
- (46) When it is time to use the instrument, turn on the power and place the reagent pickup tubes into the reagent containers, prime all reagents, perform a shut down and then perform a Start Up. Results must be within tolerance.
- (47) Perform daily QC and operate as usual.
- (48) Beginning and end of stand operations
- (49) Beginning of stand
- (50) Obtain one urine collection container or a 50-mL conical tube, seven 3 or 5-mL EDTA tubes (1 clean aspiration system, 3 reproducibility, 3 carryover), enough supplies to draw two 5-mL EDTA blood tubes, and a few plastic transfer pipettes.
- (51) Using all new containers of reagents, carefully unwrap and place all reagent pickup tubes in their appropriate container after pouring 30-mL Isoton® III into 30-mL plastic container. Use the Isoton® when performing the calibration carryover check. Handle reagent tubes by the collar only to avoid contamination. Turn the power ON. Prime reagents through all the lines by selecting DILUTER FUNCTIONS, PRIME REAGENTS, and choose ALL.
- (52) Perform the Start Up procedure. This should take approximately 5-6 minutes. Results should be within tolerance.
- (53) Contact the local Beckman Coulter representative to perform an instrument verification procedure on set up day or as soon as possible.
- (54) Calibrate, perform, and verify QC checks according to procedure.

- (55) Review and verify system set up. Select SPECIAL FUNCTIONS, SET UP, SYSTEM SET UP:
- (56) Select SHIFT
- (57) Move the cursor and set up the starting times for each shift and press [Enter] or the arrow keys. The system automatically calculates the end of the shift to prevent overlap.
- (58) Set shift 1 "Time" at 0800.
 Set shift 2 "Time" at 1230.
 Set shift 3 "Time" at 2230.
- (59) Press [F10] to save and return to the previous screen.
- (60) Select REAGENTS - Update reagent logs
- (61) To record new reagent information: Key in the new reagent information: lot number, date reagent opened (pressing [Enter] automatically gives today's date), and the expiration date. Do not forget to enter revised expiration dates where appropriate. Press [Enter] after each item. Press [F10] to save the data and leave the reagent screen.
- (62) Select INSTITUTION.
- (63) Enter the "SUPER" password. Type in:

```

Name:           NHANES
Lab Director:   Brenda Lewis
Address:        NCHS Maryland
Telephone:      301-436-7080
(Default) Instrument:  AA06042
                  AA31227
                  AB08075
  
```

Use space bar to choose YES for Name Printed on Graphic Printer and NO for all other information. Press [F10] to save.

- (64) Select COMMUNICATION DEF. Enter the SUPERVISOR PASSWORD, "super." Select, HOST COMPUTER DEFINITION.
- (65) Use [Enter] or arrow keys to move to the appropriate field.
- (66) Type in the following information where necessary and use the space bar to choose between the responses.

```

STKS 2A Host Mode  No  Enable Spooler      Yes
                  Replace Null by SPNo
Timeout (secs)    8
Baud rate         9600
Parity            none
Stop bits         1
Handshake         Yes
Block size        256

Graphic transmission
DF1              No
DF2              No
CVS histograms   No
RBC histograms   No
PLT histograms    No
  
```

- (67) Press [F10] to save and [Esc] to return to previous screen.
- (68) Select Language.
 Ensure the default is "ENG." Press [F10] to save and exit.
- (69) Select IQAP ID#.
 - Type in the IQAP number.
 - Enter 32979 1 T1 for instrument AA06042

32979 1 T2 for instrument AA31227
32979 1 T3 for instrument AB08075
Press [F10] to save and exit.

- (70) Select SET DATE/TIME.

Move the cursor and enter the correct date or time and press [Enter] or use the arrow keys. Press [F10] to save and exit.

- (71) Select LYSE VOLUME.

Press space bar to choose 1 L. Press [F10] to save and exit.

- (72) Select OPTIMIZE HARD DISK.

Type in the password "super" at the prompt and then press [Enter]. Use the spacebar to select YES. Press [F10] to save. Press [F9] to return to the main menu.

- (73) Review sample analysis set up. Select SPECIAL FUNCTIONS, SET UP, and SAMPLE ANALYSIS SET UP. Type in the password "super."

- (74) Select DISPLAY FORMATS, REPORTING UNITS.

Use the space bar to toggle to US 1. Press [F10] to save and [F9] to return to main menu.

- (75) Select PRINT OPTIONS.

Auto Print Format
Select [GRAPHIC FORMAT]

Ticket Options
Verify the following settings.
PRINT UNITS: Y
PRINT NORMAL RANGES: Y
PRINT PARAMETER LABELS: Y
TICKET PRINTER CONNECTED: N
S/D FLAGS: Y
DIFF ORDER: Standard
AUTO-TICKET PRINT: N
TICKET FORMAT START OF
NEXT FORM Continuous

Spooler Priority
Leave [AUTO PRINT] as the default.

Graphic Options
Verify the following settings.
Width/Font
Page Format Wide/Large

DF 1. RBC, PLT : Yes
DF2, DF3 : No
VCS Histograms : No
Demographics : No
S/D flags : Yes
Print Units : No
Print Diff Box : No
Print Normal Ranges : No

Optional Printer
Select "N."

- (76) Select ACTION LIMITS [Enter], DEFINITIVE FLAG LIMITS [Enter].

Disable definitive flag limits. Set low limit for all parameter values to zero except for PCT. Set lower limit for PCT to 0.000. Set high limit to 99.9 for all parameters except RBC, PLT, and PCT. Set RBC upper limit to 9.9, PLT to 999 (with no decimal) and PCT to 0.990. Press [F10] and [Enter] to save and exit.

- (77) Select ACTION LIMITS [Enter], HIGH/LOW FLAG LIMITS [Enter].

Disable High/Low flag limits. Set low limit for all parameter values to zero except for PCT. Set lower limit for PCT to 0.000. Set high limit to 99.9 for all parameters except RBC, PLT, and PCT. Set RBC upper limit to 9.9, PLT to 999 (with no decimal) and PCT to 0.990. Press [F10] and [Enter] to save and exit.

- (78) Select XB LIMITS.

Enter target and limit percent values.

XB Limits

Target	Limit %
--------	---------

MCV 88.5 3	
------------	--

MCH 29.5 3	
------------	--

MCHC	33.5 3
------	--------

Press [F10] to save and exit.

- (79) End of stand
- (80) Obtain three urine collection cups and three 5-mL EDTA blood tubes.
- (81) Review, print, and clear error file.
- (82) Select SPECIAL FUNCTIONS, ERROR FILE [Enter]. Review data and print [F4]. Document any relevant information on the print out and file in the logbook. Delete file [F8] -- "Do you want to delete error file; NO" message will appear. Use the space bar to change NO to YES [Enter]. Return to main menu [F9].
- (83) Download IQAP files to diskette following procedure in Section X.D.1.
- (84) Perform daily Shut Down. Let cleaning agent remain in the instrument for at least 30 minutes.
- (85) Perform Start-up.
- (86) Disable the reagent sensors. Select SPECIAL FUNCTIONS, DIAGNOSTIC, OPERATOR OPTIONS, FLUIDIC TESTS, and DISABLE REAGENT SENSORS. Toggle through each reagent line using the space bar to change "ON" to "OFF."
- (87) Bleach the reagent lines.
- (88) Remove all reagent pickup tubes from the reagent containers. Dispose of all open containers. Wash and save the reagent collars.
- (89) Prepare a 25% solution of Clorox bleach (250-mL) and distilled water (750 mL) in one liter bottle. Place all reagent pickup tubes into the 25% bleach/distilled water solution. Select DILUTER FUNCTIONS, PRIME, PRIME ALL. Press [Enter]. Repeat this procedure two more times.
- (90) Place all reagent pickup tubes into containers of distilled water.
- (91) Bleach the apertures and flow cell.
- (92) Mix 15 mL of Clorox bleach and 15 mL of distilled water in a plastic container.
- (93) Put 30 mL of distilled water in a second plastic container.
- (94) Open the front panel.
- (95) Select DILUTER FUNCTIONS, and DISINFECT. Press [Enter].
- (96) The instrument defaults to 15 minutes; stop the procedure after 3 minutes. Press [Enter].
- (97) Immerse the bleach probe in the bleach solution when the screen displays, "PRESS ANY KEY WHEN READY TO ASPIRATE BLEACH." Press any key. Aspirate all the bleach solution.

- (98) Immerse the bleach probe in the distilled water when the screen displays, "PRESS ANY KEY WHEN READY TO ASPIRATE WATER." Press any key. Aspirate all the distilled water. Stop the cycle after 3 minutes by pressing [F4].
- (99) Wait until the screen displays Select Function before touching any keys. When Select Function is displayed, continue with the following procedure.
- (100) Disinfect the needle. Bleach the aspiration system using the Clean Needle procedure.
- (101) Prepare a fresh bleach solution. Put one-mL Clorox bleach and one-mL distilled water into 5-mL lavender top Vacutainer tube.
- (102) Stop the system before beginning the Clean Needle procedure. If status message is not Select Function or Compressor Off, go to SAMPLE ANALYSIS, RUN SAMPLE, [F3], Run. Press [F9] Stop.
- (103) Select SPECIAL FUNCTIONS, DIAGNOSTICS, OPERATOR OPTIONS, FLUIDIC TESTS, and CLEAN NEEDLE. Press [Enter] and follow the screen instructions. Wait for green light before placing tube into the carousel.
- (104) Perform reproducibility in Primary and Secondary mode using this 1% bleach solution. Prepare a 1% bleach solution and fill one 5-mL EDTA blood tube with this solution. Turn the diff off. Is "Sample Mode?" displayed? If YES, go to step 3. If NO, press [F9] Stop. Press [F6]. Press space bar to turn the DIFF OFF. Press [Enter]. Press [F2] START PRIMARY. Select SPECIAL FUNCTIONS, CALBRATION, and REPRODUCIBILITY. Select Primary and aspirate three samples of 1% bleach. Enter [F3]; sample mode. Enter [F9] Stop. Select Secondary mode and aspirate three samples of 1% bleach solution.
- (105) Rinse the reagent lines. Make sure all reagent lines are immersed in containers of distilled water. Select DILUTER FUNCTIONS, PRIME, PRIME ALL. Press [Enter]. Repeat this procedure two more times.
- (106) Perform a Start Up. Make sure containers are full.
- (107) Perform reproducibility in Primary and Secondary mode by using distilled water. Fill one 5-mL EDTA blood tube with this distilled water. Turn the diff off. Is "Sample Mode?" displayed? If YES, go to step 3. If NO, press [F9] Stop. Press [F6]. Press space bar to turn the DIFF OFF. Press [Enter]. Press [F2] START PRIMARY. Select SPECIAL FUNCTIONS, CALBRATION, and REPRODUCIBILITY. Select SPECIAL FUNCTIONS, CALBRATION, and REPRODUCIBILITY. Select Primary and aspirate three samples of distilled water. Enter [F3], sample mode. Enter [F9] Stop. Select Secondary mode and aspirate three samples of distilled water. Press [Enter].
- (108) Select DILUTER FUNCTIONS, START-UP. Press [Enter]. Perform one additional Start-Up procedure with the lines in the distilled water containers.
- (109) Cycle the instrument dry.
- (110) Remove all reagent pickup tubes from the distilled water containers.
- (111) Continue to cycle the instrument dry by priming air through all the lines by selecting DILUTER FUNCTIONS, PRIME REAGENTS and choose ALL. Repeat these actions at least three times.
- (112) Open front cover. Using hemostats, seal tubing with check valve coming out of the bottom of the sheath tank. Loosen the four large screws that hold the panel containing the sheath tank. Gently pull panel away from instrument. Trace two red wires from top of sheath tank to plastic electrical connection. Disconnect the junction. Insert paper clip into junction so that a circuit is formed on the instrument side of the connector. Do this by bending paper clip into a U and placing one end of paper clip into each of the open sockets on the instrument side of the junction. This will allow the instrument to Drain, Vent, and Shut Down without reagent. Select SPECIAL FUNCTIONS, DIAGNOSTIC, OPERATOR OPTION, and DRAIN AND VENT. Press [Enter.]
- (113) Select DILUTER FUNCTIONS, and SHUT DOWN. Press [Enter].
- (114) After cycles are complete, remove paper clip, reconnect junction, put panel back into instrument and tighten screws, then remove hemostats.

- (115) Enable the reagent sensors. Select SPECIAL FUNCTIONS, DIAGNOSTIC, FLUIDIC TESTS, and DISABLE REAGENT SENSORS. Toggle through each reagent line using the space bar to change "No" to "Yes."
- (116) To protect the reagent pickup tubes from contamination, wrap them securely in paper towels. Allow air to continue to dry the tubes.
- (117) Turn off the power.
- (118) Rinse exterior of BSV with distilled water to remove built up Isoton and blue Clenz agent. Blot the BSV dry with lint free tissue. Do not use gauze.
- (119) Wash exterior of instrument with distilled water.
- (120) Empty waste and rinse container.
- (121) Blow air through the flow cell at the end of each stand. This is referred to as the Triple Transducer Module (TTM). Address questions to the service representative at 1-800-526-7694. Remove the 6-inch sample line to the flow cell at the mixing chamber. Connect a 10-mL syringe with tubing to the 6-inch line and force air into the line to remove ALL remaining liquid from the flow cell and the 6-inch sample line. Reconnect the sample line.

9. Beckman Coulter Reportable Range of Results

Parameter	Linearity	Limits: The greater of
WBC x 10 ³ cells/ μ L	0 to 99.9	0.2 or 3.0%
RBC x 10 ⁶ cells/ μ L	0 to 7.00	0.05 or 2.0%
Hgb g/dL	0 to 25.0	0.2 or 3.0%
MCV fL	50.0 to 150.0	2.0 or 3.0%
Plt x 10 ³ cells/ μ L	0 to 999	10.0 or 7.0%
MPV fL	5.0 to 20.0	5.0%

10. Quality Control

- (1) Latron latex controls (PN 7546914 - 5 x 16-mL) The Latron control is for use in monitoring the volume, conductivity, and light scatter (VCS) parameters on the MAXM. Use the Latron control immediately following the Latron primer. Run this control at the beginning of each day. Date and initial the vials when opened.
- (2) Principle -- The Latron control is a ready-to-use suspension of latex particles. These particles pass through the flow cell and produce characteristic electrical signals. It measures these signals as volume, conductivity, and light scatter.
- (3) Reagents -- Latex particles suspended in a buffered bacteriostatic and fungistatic medium containing a surfactant.
- (4) Storage, handling, and stability -- Sealed vials are stable until their expiration date when stored and used at 2–30°C (35–86°F). Once open, date, and initial vials. Opened vials are stable for 30 days when stored at recommended temperatures. Keep vials tightly capped when not in use.
- (5) Indications of instability or deterioration -- Inability to recover expected results might indicate product instability or deterioration due to improper storage, handling, or contamination. Discard vial if debris is visible and put new vial into use.
- (6) Instructions for use -- Before running control, verify that a Start Up has been performed.

- (7) Run Latron primer in the secondary mode. Select CONTROLS, then CONTROL RUN and press [Enter]. If the LATEX FILE does not appear, press [F2] File. Select the LATEX FILE. Press [Enter]. Press [F3] Run [F4] Primer. Cycle the primer by immersing the aspirator tip completely in the primer. Press and release the sample bar. The aspirate probe for open vials will begin to aspirate the primer. Remove vial when the audible "beep" occurs. Evaluate primer results. Results should be less than 500. Press [Esc] to remove the primer run.
- (8) Run the latex control. Mix a room-temperature control vial gently by inverting five (5) to eight (8) times. Be careful to avoid foaming. When Select Function appears on the status line, press [F3] Run, [F3] Control (SECONDARY). Cycle the control by immersing the aspirator tip completely in the vial. Press and release the sample bar. The aspirate probe for open vials will begin to aspirate the control. Remove vial when the audible "beep" occurs.
- (9) Examine the Mean Channel and Coefficient of Variation for volume, conductivity, and light scatter for the Diff Mode only. Compare the results to the Assigned Mean Values and Expected Coefficient of Variation. Check for H (high) or L (low) beside the results. If there are no H's or L's, results are within range. Press [F4] to print results for the logbook. If an H or L displays, do the following:
 - (10) Bubbles in the flow cell or improper vial handling -- Rerun the primer and control.
 - (11) Contaminated control, improperly mixed, or past open vial expiration date -- Wipe the aspirator tip. Try a new vial of Latron control. Mix gently according to directions.
 - (12) ":::::" System detects a clog in the flow cell -- Press [F3]. Press [F7] to PURGE the flow cell. Press [F4]. Cycle the Latron primer again. Press [Esc] [F3] [F3]. Cycle the Latron latex control again. If the control is still "out" of range, repeat the actions 2 or 3 more times. If the problem remains, perform a Shut-Down and Start-Up procedure. If problem continues, call a Beckman Coulter representative for assistance.
 - (13) Assigned value or range is incorrect -- Be sure the assigned values and ranges match the ones on the Latron package insert. If in error, correct them by selecting SPECIAL FUNCTIONS, SET-UP, CONTROL SET UP.
 - (14) Setting up a Latex control file

Set up a file each time a new lot number is received. Assigned channel, expected range, and %CV are automatically entered; change them to assigned values on assay sheet, if necessary.

Choose SPECIAL FUNCTIONS, SET UP, CONTROL SET-UP, LATEX FILE. Select a NOT SET-UP or an inactive file. Enter the name of the file, (Latex), Lot #, expiration date, and technician initials. Check entries. Press [F10] to save and exit.
 - (15) 5C Cell Controls Tri Pack contains Normal (blue), Abnormal I (yellow), Abnormal II (pink) (PN 7547001 - 9 x 3.3 mL.) The 5C-cell control is a hematology reference control used to monitor the performance of instruments with complete CBC and VCS differential technology. Run this control at the beginning of each session. Date and initial the open vials.
 - (16) Principle -- The 5C cell control consists of human blood so repeated measurements to monitor daily performance of the instrument system is possible.
 - (17) Reagents -- 5C-cell control consists of treated, stabilized human erythrocytes in an isotonic bacteriostatic medium. 5C-cell control also contains a stabilized, platelet-sized component, and fixed erythrocytes to simulate leukocytes.
 - (18) Storage, handling, and stability -- Store 5C-cell control at 2–8°C. When stored at 2–8°C, sealed vials are stable at least until the expiration date shown on the Table of Expected Results. Note: The MCV and/or RDW parameters may show trending through the shelf life of the product. This is inherent to the product and is not an indicator of product stability. Date and initial open vials. Opened vials are stable for 13 days or 13 events when stored at 2–8°C.

Potential biohazard -- Each human donor used in preparation of this material was tested by an FDA-approved method for the presence of the antibodies to Human Immunodeficiency Virus (HIV) and Hepatitis C (HCV) as well as for Hepatitis B surface antigen and found to be negative (were not repeatedly reactive). Handle these reagents at Biosafety Level 2 because no test method can offer complete assurance that these and other infectious agents are absent.

- (19) Indications of instability or deterioration -- Inability to obtain expected values without known instrument problems or gross hemolysis (darkly colored supernatant) indicate product deterioration. However, a slight pink color to the supernatant is normal. Do not confuse this pink color with deterioration of the product.
- (20) Instructions for use -- Remove 5C cell control tubes from refrigerator and warm to ambient temperature for 10–15 minutes. After warming, mix by hand as follows:
Do not use a rotator, rocker, or mechanical mixer. Roll the tube slowly between the palms of the hands eight full rotations in an upright position. Invert the tube and slowly roll it again eight times. Gently invert the tube eight times. Repeat these actions again.
- (21) Run the 5C-cell control in the Primary Mode of the instrument. Select SAMPLE ANALYSIS, RUN SAMPLES. Press [Enter]. Is "SAMPLE MODE?" displayed? If no, press [F9] STOP. Make sure the DIFF is on. If it is off, press [F6]. Press [F2] START PRIMARY. Complete the entire procedure and return the controls to the refrigerator within 30 minutes.
- (22) Identify the sample by holding the bar code label in front of the reader. Place the control in the carousel. Repeat for other levels of controls.
- (23) Check the results of each control. Select CONTROLS, REVIEW, OR REPORT and [F2] to select a file for review. Choose one control file and [Enter]. Check for H (high) or L (low) beside the results. If there are no H's or L's, results are within range. To print results for the logbook for the last control run, press [F4] Print. Use [F2] to select another file and repeat actions. Review and print reports for all three controls and file in the logbook. If a H or L displays, consider the following reasons and perform and document the following actions:
- (24) Improper mixing -- Follow instructions and rerun control.
- (25) Control files set-up incorrectly -- Make sure the assigned values and ranges match those on the control package insert. If in error, correct them by selecting, SPECIAL FUNCTIONS, SET UP, CONTROL SET UP.
- (26) Chance (statistical outlier) -- Rerun the control. If it is still out, continue with the following actions.
- (27) Change in the control -- Try another vial or level of control.
- (28) Instrument change -- Watch for normal sample flow. Call Beckman Coulter representative to help troubleshoot abnormal operation.
- (29) Setting up a CBC/Diff control file
Set up a file whenever receiving a new lot number of control. Enter control package insert information for control with differential. Enter information using the bar code wand. Enter information manually if necessary.
Choose SPECIAL FUNCTIONS, SET UP, and CONTROL SET-UP. Select CBC DIFF/FILE. Select a NOT SET-UP or an inactive file.
- (30) Using bar code scanner
Scanning bar code units -- Choose the correct section of the package insert to scan making sure to select the part with the correct Unit Format (US), for the correct instrument (MAXM).
- (31) Hold the wand like a pencil.
- (32) Place the wand in section 1, Lot No. Read this section first.
- (33) Gently slide the wand back and forth over the bar code until the instrument beeps. The lot number is now entered. Start and end each scan in the white space.
- (34) Beckman Coulter's expected ranges appear automatically when the control lot number is entered in an empty file.
- (35) Follow the same procedure for the expiration date and assigned values. (Values 2-10)
- (36) Manually select and enter Shift and Operator ID.
Check all entries to make sure they are correct. Press [F10] to save and exit.
- (37) Manual entry

Enter lot number. This enters the IQAP number automatically. Enter the expiration date, shift, and operator code. Continue to enter the package insert data. Press [Enter] after each entry. Press [↓] at the end of each row. The system automatically enters the level and default ranges based on the first two digits of the lot number. Check to make sure all entries are correct. Press [F10] to save and return to the previous screen.

(38) Control statistics and graphs

(39) Use to review and print:

Control results, cumulative statistics and graphs for CBC/DIFF files or
Control results, cumulative statistics and histograms for LATEX Files or
Check cumulative results for trends or shifts as necessary for troubleshooting.

Print, review, and delete cumulative statistics and graphs for all CBC/DIFF and LATEX files at the end of each stand after transferring information to the IQAP disk and file in the logbook.

(40) CBC/DIFF control review and report

(41) Use this report to review and print control results and cumulative statistics for CBC files. Select CONTROLS, REVIEW OR REPORT, and [Enter]. Press [F2] to select one of the following files, Normal, Abnormal I, Abnormal II, and Latex [Enter]. Press [F4] to print. Review and file in logbook. Continue to select each file in turn, print, and file.

Screen-specific function keys:

[F2] File Displays all available files. Use ↓ and ↑ to select the correct file. Press [Enter].

[F4] Print Prints the entire control file in a line list format on 8.5 x 11 in paper.

[F6] Remove/Restore

Removes a highlighted run from the calculations. A DEL appears in place of the run number. The statistics recalculate. Pressing [F6] restores the run and original statistics.

[F8] Delete File

Deletes the current control file. Displays a message "You have asked to delete ENTIRE control file. Are you sure you want to delete?" No. Press the space bar to select Yes or No.

[←] [→] Press [←] or [→] to see parameters not currently displayed on the screen.

[F9] Menu Displays the main menu.

(42) Latex control review and report

(43) Use this report to review and print control results and cumulative statistics and histograms for LATEX files. Check cumulative results to look for trends, shifts, or, if necessary, troubleshooting.

(44) Screen-specific function keys:

Press [←] To go back to DIFF Latex Control Review

[F2] File Displays all available files. Use ↓ and ↑ to select the correct file. Press [Enter].

[F4] Print Prints the entire control on a graphic printer.

[F6] Remove/Restore

Removes a highlighted run from the calculations. A DEL appears in place of the run number. The statistics recalculate. Pressing [F6] again restores the run and original statistics.

[F8] Delete File

Deletes the current control file. Displays a message "You have asked to delete ENTIRE control file. Are you sure you want to delete?" No. Press the space bar to select Yes or No

[←] [→] Press [←] or [→] to see parameters not currently display on the screen.

[F9] Menu Displays the main menu.

(45) Interlaboratory Quality Assurance Program (IQAP)

All instruments participate in Beckman Coulter's IQAP program. The IQAP program includes saving results of 5C Cell controls, transferring them to a disk, and sending them to

Beckman Coulter, who compares the results to other laboratories. Beckman Coulter issues a report that contains a statistical analysis to evaluate performance. Perform this procedure at the end of each stand, or when a lot expires.

Follow procedure for CBC/DIFF and Latex control review and report. After downloading and printing results, delete control files [F8].

- (46) Procedure to download IQAP files to diskette.
- (47) Slide the diskette into the drive with the arrow side up and pointed toward the drive in the Beckman Coulter Data Management System. Gently push the diskette until it clicks into place.
- (48) Turn the computer OFF. Wait 15 seconds, and then turn the computer back ON. A screen will appear showing the Beckman Coulter Corporation logo. To exit this screen, follow the instructions on the bottom left portion of the screen, and press [Esc].
- (49) The screen will indicate the process the system is performing. Read the instructions on the screen. When the process is complete, the DMS displays the list of possible files on the screen for review and selection. If some of the values are incorrect, edit or delete the fields on this screen. Any alteration to this screen will affect only data on the IQAP diskette, not in the MAXM computer.
- (50) FILE_NAME indicates the files by file number for downloading to the diskette.
 - LOT_# indicates the type of control. Prefixes 86, 87 and 88 refer to 5C Cell Controls.
 - SHIFT refers to the shift designation selected for running the controls. Edit this field if necessary. Use arrow keys to position.
 - START and END indicate beginning and end dates for each file. These fields are not editable.
 - REPORT indicates whether IQAP will issue a report on the lot number.
 - N_REC indicates the number of records found in the file.
- (51) Press [Esc] to leave this screen. If the IQAP# and Serial# are present and valid, the following screen message will appear, "Updating the IQAP Disk." After writing the control files to the diskette, a screen message will give instructions to follow. Follow these instructions and the computer will reboot to the routine program. To remove the diskette, press the eject button on the lower right of the diskette drive opening. Do not remove the diskette until the drive indicator light is off.
- (52) Label the diskette with the supplied IQAP ID label. Place the diskette in pre-addressed mailer provided and mail. Do not place the label over the drive spindle or high-density hole.
- (53) X_B analysis
- (54) X_B analysis is a QC method monitoring instrument performance by tracking the MCV, MCH, and MCHC parameters of SP samples. These indices are very stable and show little variance between SP samples. This method displays the moving average of SP samples and compares it to established target values. Target values are MCV = 88.5, MCH = 29.5, and MCHC = 33.5. Review and print the X_B current batch at the beginning on each session when printing the 5CCell control results. Review, print, and file the Coulter X_B current batch at the beginning of each session when printing the 5CCell control results.
- (55) X_B batch means:

Use to view the calculated means for the last batch of 20 SP results. The MAXM calculates MCV, MCH, and MCHC means. Review, print, file, and delete the Batch Means at the beginning of each week when performing the weekly laboratory QC and at the end of each stand. Evaluate the percentage difference from the target limit to determine whether the batch is in or out of control. Select SAMPLE ANALYSIS, X_B , and CURRENT X_B BATCH. Press [F2] [F4]. Print and file in logbook.

To change the status of X_B , select [F5] Optns on the RUN SAMPLES screen.

Screen-specific function keys:

- [F4] Print Prints the table.
- [F6] Delete Last Batch
Deletes the last batch. Delete only if the batch is badly skewed because of non-random sampling or an instrument problem that it will adversely affect many later batches.
- [F8] Delete Table
Deletes the entire table.
- [←] [→] Press [←] or [→] to see parameters not currently displays on the screen.
- [F9] Menu Displays the main menu.

(56) X_B Graphs

Review, print, file, and delete the Batch Means and graphs at the beginning of each week when performing the weekly laboratory QC and at the end of each stand. Select SAMPLE ANALYSIS, XB, XB GRAPHS, and [F4] to print. File in the logbook.

(57) Proficiency testing

Evaluation and participation in the College of American Pathologist (CAP) proficiency-testing program is part of the comprehensive quality control program. These survey materials are shipped three times per year and consist of 5 3-mL whole blood specimens. Follow all CAP instruction in preparing the materials before performing the test. Run specimens in a manner identical to routine specimens. Fill out the CAP result form, make a copy for the logbook, and send results to CAP.

(58) Linearity for WBC, RBC, Hgb and Plt parameters

Lin-C(PN 7547065 - 5 x 3.3-mL) The Lin-Clinearity controls verifies the reportable range of Beckman Coulter hematology systems that use both Isoton III diluent and Lyse S III diff lytic reagent.

OR

CAP Hematology Calibration Verification/Linearity Survey (LN9) - These materials are shipped twice per year and consist of 18 3-mL liquid specimens. Follow all CAP instruction in preparing and running the materials before performing the test. Fill out the CAP result form, make a copy for the logbook, and send results to CAP.

Run either the Lin-Cor CAP LN9 survey material:

- At installation
- At least yearly
- Whenever experiencing an altitude change of 1 mile or more between stands

(59) Principle - Lin-Clinearity controls are human blood components from which repeated measurements verify the reportable range of Beckman Coulter hematology systems. Controls contain one each of Ultra Low Range, Low Range, Mid Range, High Range, and Ultra High Range. Lin-Cverifies ranges for the following parameters: WBC, RBC, Hgb, and Plt.

To ensure the accuracy of linearity control ranges, Beckman Coulter system calibrates with S-CAL calibrator.

(60) Reagents -- Lin-Ccontrols consist of treated, stabilized, human erythrocytes in an isotonic bacteriostatic medium. Linearity controls also contain a stabilized platelet-sized component, and fixed erythrocytes to simulate leukocytes.

(61) Storage, handling, and stability -- Ship Lin-Ccontrols in thermally insulated containers designed to keep kits cool. Store Lin-Clinearity controls between 2–8°C (35–46°F). Store vials in an upright position to achieve maximum product yield. Storage of the control product in a cap down position might require additional mixing or complete resuspension of cellular components.

Potential biohazard -- Each human donor used in preparation of this material was tested by an FDA approved method for the presence of the antibodies to Human Immunodeficiency Virus (HIV) and Hepatitis C (HCV) as well as for Hepatitis B surface antigen and found to be negative (were not repeatedly reactive.) Handle these reagents at Biosafety Level 2

because no test method can offer complete assurance that these and other infectious agents are absent.

- (62) Indications of instability or deterioration -- Inability to obtain expected values without known instrument problems or gross hemolysis (dark colored supernatant) indicates product deterioration. A slight pink color to the supernatant is normal. Do not confuse this pink color with deterioration of the product.
- (63) Instructions for use -- Remove Lin-Ccontrols from refrigerator and warm to ambient temperature for 10–15 minutes. After warming mix by hand as follows:

Do not use a rotator, rocker, or mechanical mixer. Roll the tube slowly between the palms of the hands eight full rotations in an upright position. Invert the tube and slowly roll it again eight times. Gently invert the tube eight times. Repeat these actions again. Controls expire 7 days after opening.
- (64) Turn the blood detectors off to analyze the Ultra Low Range. Select SPECIAL FUNCTIONS, DIAGNOSTICS, OPERATOR OPTIONS, BSV TESTS, and BLOOD DETECTOR ON/OFF. Press [Enter]. Use space bar to select ON or OFF. Press [Enter].
- (65) Disable the differential before analyzing Lin-Clinearity controls. Is “SAMPLE MODE?” displayed? If no, press [F9] STOP. Make sure the DIFF is off. If it is on, press [F6].
- (66) When analyzing Lin-C linearity controls, flags such as L, LL, H, HH, R, RR, *, *R, and *V will occur. Ignore these flags if a numerical result is obtained. When ---- (voteout), (incomplete computation) or +++++ (over range) flags occur, the sample should be repeated. Ignore Beckman Coulter histogram differential flags and results.
- (67) Run the Lin-C controls in the Primary Mode of the instrument. Select SPECIAL FUNCTIONS, CALBRATION, and REPRO-DUCIBILITY. Press [Enter]. Press [F2] START PRIMARY. Analyze controls six times and record the results for WBC, RBC, Hgb, and Plt parameters for each sample on the worksheet. Repeat flagged samples with non-numeric results.
- (68) Beckman Coulter recommends that five sample results should be used to calculate the mean value. Use a minimum of three samples to calculate the mean when repetitive results with non-numeric values occur. Delete the first sample result. Plot mean recovered values on the Lin-C linearity control graphs.
- (69) Compare the mean value to the linearity control ranges listed on the Table of Expected Results on the package insert. Mean values should recover within the ranges. Use the ranges established by Beckman Coulter Corporation as a guideline.
- (70) Plot the instrument background count as a zero value to extend the reportable range. Beckman Coulter will prepare tabular summaries and graphic presentations of the data. Submit the top copy of the worksheet to IQAP at the following address:

Beckman Coulter Corporation
IQAP (M/C 31 B04)
P.O BOX 169015
Miami, FL 33116-9015

11. Interpretation of Results and Remedial Action

- A. Sample Person hemoglobin and hematocrit review and remedial actions.
- B. Review all results to make sure the hemoglobin and hematocrit are acceptable. The hematocrit should be approximately three times the hemoglobin.

Run samples in duplicate whenever possible. If the whole blood is insufficient, it is acceptable to average and save only one run. To save results where only one run is possible, use the mouse to direct the mouse arrow to the Average Selected ID box and left click. A warning message text box displays that states, "You have downloaded only one (1) run from the CoulterMAXM for SPID XXXXXX" and asks, "Do you want to create an average based on a single run?" To record a "Yes" response, use the mouse to the Yes button and left click or type [Y/y]. To record a "No" response, use the mouse to the No button and left click, or type [N/n], or press [Enter]. If a Yes response is recorded, the CBC results display in the bottom Results section of the window. A "No" response cancels the action and returns the window to its previous state.

For results where only one run was possible, the Comment box at the bottom of the window defaults to "result based on single run."

- C. Running samples in duplicate.

Run all samples in duplicate and average the data. Whenever a sample is run in duplicate, both results display in the SP Data section. To average these two results, use the mouse to Average ID#XXXXXX button and left click.

The difference between duplicate values for WBC, RBC, Hgb, MCV, Plt, NE#, LY#, MO#, EO# and BA# are calculated and evaluated to determine if the differences are within the following CDC established precision limits.

CDC-established precision limits:

Test	Precision Limits
WBC	0.4×10^3
RBC	0.1×10^6
Hgb	0.4 g/dL
MCV	2.2 fL
Plt	23×10^3
NE#	0.4×10^3
LY#	0.2×10^3
MO#	0.2×10^3
EO#	0.2×10^3
BA#	0.2×10^3

If RBC parameters are out of range, evaluate XB for a drift. If drift is evident, evaluate the possibility of an instrument malfunction. If any WBC differential absolute number is out of range, check the WBC scattergram for abnormal cell population(s).

Evaluate and save results when precision limits are not exceeded.

Evaluate results for all parameters. Evaluate each parameter for error, overlimit, and precision checkmarks, and "H" or "L" action limit and reference range flags. If there are no checkmarks in the Precision column (no precision limit was exceeded), save the results to the database. To save the results to the database, use the mouse to the Save button and left click or type [Shift] [S/s]. After results are saved, they are erased from the screen. To

exit without saving the result to the database, use the mouse to the Close button and left click or type [Shift] [C/c].

Average, evaluate, and rerun specimens that exceed precision limits.

Average the two results by using the mouse to direct the mouse arrow to the Average ID#XXXXXX button and left click. If at this point, precision limits are exceeded for any individual parameter, the technologist is prompted to run a control and evaluate the control to determine if all control values fall within the control's established range.

If any parameter exceeds its precision limit, the Hematology Control Run window displays.

The Hematology Control Run window indicates that the "Samples for SP ID XXXXXX are outside the defined precision limits." It instructs the technologist to "Please run a successful control for Session ID XXXXXX, and then run another sample for SP ID XXXXXX." Run any one level of 5CCell control and evaluate the results. Respond to the two questions, "Did you run a control run?" and "Were all control values within established range?" To confirm these actions, use the mouse to direct the mouse arrow to each of the two check boxes and left click to record the check marks.

Save the responses to the check box questions.

Evaluate each parameter for error, overlimit, and precision checkmarks, and "H" or "L" action limit and reference range flags. Use the scroll bar to view all results. When there are checkmarks in any of the Precision columns, meaning that the precision limit was exceeded, rerun the blood sample a third time. If there is insufficient blood to run the CBC a third time, do so and save the result to the database.

Run the blood sample a third time. Whenever a sample is run more than once, all results in the SP Data section are displayed. To re-average these results, use the mouse to direct the mouse arrow to Average ID#XXXXXX button and left click. The initial averaged result for parameters that do not exceed precision limits are fixed and are not recalculated.

Override the current averaged results for parameters that exceeded the precision limit with a new average.

The first three runs are recalculated to find the closest two results for parameters where the difference between any two results exceeded its precision limits. If all parameters now meet precision limits (there are no checkmarks in the Precision column), save the results to the database.

If necessary, run the specimen a fourth time and evaluate.

If precision limits are still not met for any parameter, run the blood tube a fourth time. Re-average, evaluate, and save the results. If after four runs precision limits are still not met for any parameter, a comment is automatically attached to the results. Do not run a specimen more than four times.

In general, run one SP in duplicate through the Coulter average the result, and save the data to the database. The Hematology module will display data for multiple SPs in the SP Data section. Each individual SP's results or pairs of results are displayed in a different color.

Evaluate each parameter for error, overlimit, and precision checkmarks, and "H" or "L" action limit and reference range flags. If there are no checkmarks in the Precision column (no precision limit was exceeded), save the result to the database. To save the result to the

database, use the mouse to the Save button and left click or type [Shift] [S/s]. To exit without saving the result to the database, use the mouse to the Close button and left click or type [Shift] [C/c]. If there are any Precision checkmarks, run a 5C Cell control, evaluate the control results, rerun the blood tube a third time, re-average, evaluate, and save the results.

- D. MAXM parameter codes
- E. Review the message

The Beckman Coulter MAXM uses triplicate counting with strict voting criteria. It has proprietary flagging algorithms to confirm parameter results before reporting. The instrument may not detect a transient or partial aperture blockage by any of these processes. A partial aperture blockage may cause erroneous results, such as WBC count lower than what is present. Monitor the aperture-viewing screen when cycling specimens that are likely to contain fibrin or debris.

The MAXM displays abnormal parameter results for all cell populations and values. The following table describes the display and cause for the parameter codes.

Display	Cause
All Parameters	
"●●●" for a single parameter result	Incomplete or abnormal computation
"●●●" for all parameter results and PART.ASP message displayed	Sample integrity check failed, short sample or clot
"-----" for CBC parameter results, and no average histogram for the affected parameter If WBC is "-----", then LY#, MO#, NE#, EO# and BA# are "●●●" If RBC is "-----", then MCH, MCHC, Hct are "----_"	Total voteout (none of the three counts agreed); Diff parameters do not vote out
"*V"	Single-count period voteout; May indicate an erroneous result due to aperture blockage or lack of mixing bubble Appears next to parameter derived from the parameter with the single count period voteout
"+++++" for parameter results IMPORTANT If the WBC, RBC, Hgb, or Plt result is +++++, run a blank cycle to prevent carryover to the next sample.	Result exceeds: WBC $\geq 100.0 \times 10^3$ cells/ μ L RBC $\geq 10.00 \times 10^6$ cells/ μ L Plt $\geq 1000 \times 10^3$ cells/ μ L MCV ≥ 1000 If WBC is over range an R appears next to the RBC, Hgb, Hct, MCV, MCH, MCHC, RDW, MPV, Plt and Diff % results. Results for Diff% are dots (.....). If RBC is over range an R appears next to Hct, MCH, and MCHC results. If Hgb is over range an R appears next to MCH, and MCHC results. If MCV is over range an R appears next to Hct, MCHC, and RDW results.

"+++++" for parameter results IMPORTANT If the WBC, RBC, Hgb, or Plt result is +++++, run a blank cycle to prevent carryover to the next sample.	If Plt is over range an R appears next to WBC, Hgb, MCH, MCHC, diff# and Pct results. Data Base Query displays average values for over range WBC, RBC, or Plt. [F3] Results or [F3] Edit, beneath the definitive flag section. These values are for review only; do not report them.
	IMPORTANT Sample dilution may result in erroneous differential results. Set DIFF to OFF before cycling the diluted sample when WBC is over range.
"????" for Diff results	Questionable data
Plt Parameter	
"R" next to Plt and MPV result	PDW >20% mode not between 3 and 15 fL, or non-positive curve detected, or Plt <20,000, or Total voteout of fitted curve, or WBC is over range.
RBC Parameters	
"R" next to RDW result	Excessive asymmetry in RBC histogram, or WBC or MCV over range.
"*R" next to MCV; also "R" next to RBC, Hct, MCH, MCH, RDW, Plt, and MPV	MCV below lower limit of <50 fL
WBC Parameters	
"*R" next to WBC; also "R" next to Diff numbers	Check of WBC lower threshold failed. Particles are present at the 35 fL threshold (Plt clump, giant Plt, nrbc, lyse resistant RBC, malarial parasites)
"R" next to Diff percentages and numbers	Low differential count statistics. These messages occur also: Population message: ABNORMAL WBC POP Suspect message: REVIEW SLIDE
":::" for Diff results	System detects a clog in the flow cell. There are three types of clogs: FC = Full Clog PC1 = Partial Clog 1 (partial clog in the flow cell or sample line) PC2 = Partial Clog 2 (partial clog in the sheath system)

F. Remedial action

Review the result for the affected parameter. Rerun the specimen if any of the parameter flags occurs.

G. MAXM suspect messages

H. Suspect messages flag an abnormal cell distribution or population. The system generates these messages according to an internal algorithm. Specific suspect messages indicate some abnormalities that exhibit characteristic cluster patterns. Suspect messages indicate the possibility of a particular abnormality. Not every atypical scatterplot has a corresponding suspect message.

I. Remedial action -- Rerun the specimen.

J. Physician review.

- K. The MEC physician reviews and interprets all CBC results. Results outside action limits flash and transmit to the physician immediately. The physician determines if referral for the SP for treatment is necessary.

12. Limitations of Method: Specimen Rejection, Interfering Substances and Condition

This method limits samples to human whole blood.

- A. Specimen rejection
- B. Reject clotted specimens and recollect.
- C. Interfering Substances and Conditions

Because the Coulterdirectly measures RBC, WBC, Hgb, and Diff %, it is most important to concentrate on analytes and substances that interfere with these parameters. The Coultercalculates HCT, MCH, MCHC, and DIFF # parameters. The Coulterderives MCV, RDW, PLT, and MPV from RBC or platelet histograms. The following are possible interfering substances or conditions:

- Abnormal BUN, glucose or sodium levels could affect the MCV.
- Abnormal WBCs could affect lymphocytes, monocytes, and granulocytes.
- Abnormally small WBCs could affect white count, lymphocytes, monocytes, and granulocytes.
- Clumped platelets could affect white count, lymphocytes, monocytes, granulocytes, RBC, MCV, RDW, platelet count, and MPV.
- Cryofibrinogen and cryoglobulin crystals could affect white count, lymphocytes, monocytes, granulocytes, RBC, hemoglobin, platelet count, and MPV.
- An elevated WBC count could affect RBC, hemoglobin, MCV, RDW, platelet count, and MPV parameters.
- Fragile WBCs could affect white count, lymphocytes, monocytes, granulocytes, platelet count, and MPV.
- Giant platelets could affect white count, lymphocytes, monocytes, granulocytes, RBC, MCV, RDW, platelet count, and MPV.
- Hemolyzed specimens could affect RBC, hemoglobin, platelet count, and MPV.
- Lipemic specimens could affect MCV.
- Severely icteric plasma causes increased hemoglobin. Evaluate CBC result carefully and report all parameters except the hemoglobin result
- Nucleated RBCs could affect the white count, lymphocytes, monocytes, granulocytes, and hemoglobin values.

13. Reference Ranges

- A. Males

	1-5	6-18	19-65	66+
WBC (x 10 ³ μL)	4.3-14.1	3.7-11.9	3.9-12.1	3.9-12.3
RBC (x 10 ⁶ μL)	4.00 - 5.30	4.10-5.60	4.10-5.80	4.00-5.60
Hgb (g/dL)	10.5-13.7	11.5-16.3	12.7-17.1	11.0-16.8
HCT (%)	31.8-40.8	34.4-48.3	38.0-50.3	33.1-50.2
MCV (fL)	67.6-88.2	74.3-93.0	78.1-99.2	78.9-101.4
MCH (pg)	21.7-29.8	24.3-31.7	25.7-33.8	25.6-34.4
MCHC (g/dL)	31.5-35.0	31.9-35.1	32.0-35.3	31.8-35.1
RDW (%)	11.7-16.5	11.7-14.3	11.8-15.3	12.1-16.6
Plt (x 10 ³ μL)	224-568	194-477	157-414	138-407
MPV (fL)	6.3-9.3	6.6-9.9	6.8-10.4	6.7-10.6
LY (%)	24.5-70.0	20.0-56.6	17.8-51.8	13.0-48.3

MONO (%)	0-12	0-12	0-12	0-13
NE (%)	21.4-70.5	33.2-74.7	39.7-77.3	44.7-81.9
EO (%)	0-10	0-11	0-8	0-8
BA (%)	0-2	0-2	0-2	0-2

B. Females

Age in Years	1-5	6-18	19-65	66+
WBC (x 10 ³ μL)	4.2-13.6	4.0-12.4	3.9-12.5	4.0-12.3
RBC (x 10 ⁶ μL)	3.90 - 5.20	3.90-5.20	3.70-5.20	3.50-5.20
Hgb (g/dL)	10.5-13.7	11.1-14.7	10.4-15.2	10.5-15.5
HCT (%)	32.0-40.6	33.4-43.4	32.0-45.0	32.0-46.0
MCV (fL)	69.0-88.6	74.5-93.9	73.4-98.3	79.7-100.2
MCH (pg)	22.3-30.1	24.2-31.8	23.2-33.3	25.9-33.8
MCHC (g/dL)	31.8-35.1	31.7-35.0	31.4-35.1	31.6-35.0
RDW (%)	11.4-15.4	11.6-14.8	11.8-16.6	12.0-16.1
Plt (x 10 ³ μL)	229-581	202-476	172-453	147-440
MPV (fL)	6.3-9.2	6.7-10.0	6.9-10.7	6.8-10.8
LY (%)	24.9-70.5	18.7-58.5	17.8-52.8	15.7-51.1
MONO (%)	0-12	0-12	0-12	0-13
NE (%)	21.3-69.3	32.9-77.2	39.6-77.8	42.0-79.8
EO (%)	0-10	0-11	0-8	0-8
BA (%)	0-2	0-2	0-2	0-2

C. Reference ranges for normal values were calculated from NHANES III data set using 95% reference interval(s) determined nonparametrically, through ranking the observations and determining the lower (2.5th percentile) and the upper (97.5th percentile) reference limits. The nonparametric (ranking) method was used because most measured hematology parameters have a skewed, non-Gaussian distribution.

14. Action Limits

Action limits are a guide to inform the physician that a CBC result(s) is/are abnormal. Since all specimens are run in duplicate, there is no reason to retest the sample.

- WBC male and female (all ages) < or = to 3 x 10³ μL or > or = to 16.0 x 10³ μL
- Hgb male and female (<6 years) <6.5 g/dL or >14.5 g/dL
- Hgb female (>6 years) <6.5 g/dL or >16.0 g/dL
- Hgb male (>6 years) <6.5 g/dL or >18.0 g/dL
- PLT male and female (all ages) <50 x 10³ μL or >800 x 10³ μL

Possible causes of abnormal parameters:

- High RBC, Hgb, or HCT -- dehydration, polycythemia, shock, chronic hypoxia
- Low RBC, Hgb or HCT -- anemia, thalassemia and other hemoglobinopathies
- Low MCV -- microcytic anemia
- High MCV -- macrocytic anemia, liver disease
- Low WBC -- sepsis, marrow hypoplasia
- High WBC -- acute stress, infection, malignancies
- Low platelets -- risk of bleeding
- High platelets -- risk of thrombosis

15. Specimen Storage and Handling during Testing

- A. Specimen storage
- B. Store specimens capped and place on a rocker at room temperature until processed.
- C. Run within 24 hours of drawing.

16. Alternative Method for Performing Test or Storing Specimens if Test System Fails

There is no alternative method for this test. Store EDTA tube at room temperature for no more than 24 hours. Restore the instrument to functionality and then run the specimen.

17. Test Results Reporting System: Protocol for Reporting Action Limits

Results outside the action limits are automatically brought to the physician's attention for a decision as to "course-to-follow."

18. Specimen Accountability and Tracking

All records, including QA/QC data will be maintained for 6 years. Use only numerical identifiers for SP results.

19. Quality Control Summary Statistics and Graphs

Chapter 10 in the Laboratory Procedures Manual (April 2001) includes a separate detailed description of the comprehensive quality control plan. Monitor 5C Cell control results for bias and maintain results for the entire study period. Compare all three instruments CAP proficiency results.

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