

DEPMEDS LABORATORY PROCEDURES
DEPARTMENT OF CLINICAL SUPPORT SERVICES
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL
FORT SAM HOUSTON, TEXAS 78234-6137

MCCS-HCL STANDING OPERATING PROCEDURE 01 Novemeber 01

COMPLETE BLOOD CELL COUNT (CBC) BY QBC II

1. PRINCIPLE:
 - a. The QBC calculates WBC counts, platelet counts, hematocrits, % granulocytes, % lymphocytes/monocytes, and absolute counts of lymphocytes/monocytes. This provides information concerning the oxygen carrying capability of hemoglobin, vascular integrity, and the presence of infection in the body.
 - b. A QBC capillary tube containing a specific gravity float and acridine orange dye is filled with anticoagulated (EDTA) blood and centrifuged. The specific gravity float separates the various layers of the buffy coat which are measured quantitatively by their lengths. The acridine orange dye is absorbed by the various layers of the buffy coat. Each component fluoresces at a different wavelength allowing visual marking of each component quantitatively with L.E.D. readout.
2. SPECIMEN: EDTA anticoagulated blood not more than 4 hours old and at least 2/3 full, for parameters other than platelets. For platelet counts, centrifuge specimen within 90 minutes.
3. REAGENTS AND EQUIPMENT:
 - a. Centrifuge: Calibrate speed once a year at 12,000 RPM and centrifuge timer for 5 minutes.
 - b. Venous Blood Pipetter: Calibrate twice a year for a volume of 111.1 uL.
 - c. Venous Blood Tubes: Store at 60-90°F (16-32°C); protect from moisture, direct light, and heat.
 - d. Venous Calibration Tube.

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- e. Capillary Calibration Tube.
- f. QBC Analyzer: Perform daily calibration checks.
 - (1) Press the power switch of the QBC II Reader to the (On) position and monitor the self-test sequence.
 - (2) Place the reader in the "VEN" (Venous) mode and insert the venous calibration check tube.
 - (3) Press the CLEAR button to clear any codes and the figure 8 light pattern from the LED read outs.
 - (4) Align the 1st interface (black to pale green) with the reticle arrow and press "ENTER."
 - (5) Continue reading the 2nd through the 6th interfaces in the same manner.
 - (6) Record the venous calibration check values displayed for each of the seven parameters on quality control graph or log book, then remove the tube.
 - (7) Select the "CAP" (Capillary) mode, insert the capillary calibration check tube and repeat the reading procedure, including the 7th interface.
 - (8) Record the capillary calibration check values displayed for each of the seven parameters on quality control graph or log book.
 - (9) Compare both sets of values (Ven and Cap) with the reference values and tolerances recorded on the package inserts of the calibration check tubes.

4. QUALITY CONTROL:

- a. Initial instrument QC.
 - (1) Draw 2 EDTA tubes from a normal patient.

NOTE: Use of Personal Protective Equipment (PPE) and universal precautions is required when handling biological specimens.

- (2) Spin one tube 15 minutes at 3,300 RPM for platelet- poor plasma.

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- (3) Remove and save all plasma from sample without disturbing buffy coat.
- (4) Mix packed blood cells and buffy coat
- (5) Label 3 tubes: 1, 2, and 3.
- (6) To each tube add 8 drops of platelet-poor plasma.
- (7) To tube #1 add 4 drops of packed red blood cell suspension.
- (8) To tube #2 add 7 drops of packed red blood cell suspension.
- (9) To tube #3 add 10 drops of packed red blood cell suspension.

<u>Sample</u>	<u>Target Hct</u>	<u>Platelet-poor plasma</u>	<u>Blood</u>
1	25-30%	8 drops	4 drops
2	35-40%	8 drops	7 drops
3	45-50%	8 drops	10 drops

- (10) Mix all samples well.
- (11) Perform centrifugal microhematocrit determination on each sample.
- (12) Perform QBC determination on each sample.
- (13) Hct performed by QBC should be +0.5 to +2.0% higher than the spun microhematocrit.

b. Second EDTA tube will be run as the first sample and repeated every 10th sample thereafter as an "In run" control.

c. In run" control results should agree within:

<u>Test</u>	<u>%</u>
Hct	1.5
WBC	5.5
Plt	10.0

d. Every 4 hours draw a fresh normal patient sample, confirm Hct with both QBC and microhematocrit procedures, and use sample as an "in run" control.

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5. PROCEDURE:

a. Sample preparation.

- (1) Insert the end of a QBC venous capillary tube nearest red line into pipetter.
- (2) Mix blood, then aspirate specimen. Wipe tube with gauze, ensuring tip of tube is not touched.
- (3) Press distal end of tube into closure. Remove tube from the pipetter and twist closure to ensure seal.
- (4) Roll the tube at least 10 times between fingers to mix blood.
- (5) Slide tube over the plastic float. Gently push until float is inside the tube.
- (6) Place tube into centrifuge and balance. Record the tube number with its position.
- (7) Install rotor cover, close lid and centrifuge (centrifuge preset for 5 minutes).
- (8) Promptly remove tubes from centrifuge and check that the plasma level in the spun tube is between the two red lines.

b. Analysis.

- (1) Select desired mode (Ven or Cap).
- (2) Insert the tube into the reader.
- (3) Move the tube inward until the reticle arrow is at the interface between the green closure and the bottom of red cells (1st reading).
- (4) Press ENTER. The white tube lamp will light.
- (5) Move the tube inward until reticle arrow is at bright edge of float between

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dark red and light red layers (2nd reading).

- (6) Press ENTER. The white tube lamp will turn off.
- (7) Move tube inward until the reticle arrow is at the interface between the light red and orange-yellow layers (3rd reading)
- (8) Press ENTER.
- (9) Move the tube inward until the reticle arrow is at the interface between the top of the orange-yellow layers and the bottom of the dark band (4th reading).
- (10) Press ENTER.
- (11) Move the tube inward until the reticle arrow is at the interface between bright green and pale yellow layers (5th reading).
- (12) Press ENTER.
- (13) Move the tube inward until the reticle arrow is at the interface between pale yellow layer and translucent green plasma (6th reading)
- (14) Press ENTER. In venous mode record the seven hematology values.
- (15) "CAP" mode only (7th reading).
 - (a) Move the tube inward until the reticle arrow is at the meniscus of translucent green plasma column (7th reading).
 - (b) Press ENTER. Record the six hematology values for the venous mode.

6 RESULTS:

a. Normal Values.

- (1) Hct: Males 40.0 – 52.0%
Females 36.0 - 48.0%

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(2) Plt: 150 – 400 x 10⁹/L

(3) WBC: 4.0 - 11.0 x 10⁹/L

(4) Gran: 44.2 - 80.2%.
2.0 - 8.8 x 10⁹/L

(5) Lymph/Mono: 28.0 - 48.0%
1.2 - 5.3 x 10⁹/L

b. Critical values.

	Less than	Greater than
(1) Hct:	<18%	>61%

(2) Plt:	<39	>910 x 10 ⁹ /L
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(3) WBC:	<2.0	>40.0 x 10 ⁹ /L
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c. Hcts with values less than 30% should be rechecked by microhematocrit method.

d. A differential should be performed on WBC counts less than 3 x 10⁹/L and greater than 15 x 10⁹/L.

e. Granulocytes with a absolute count less than 4 x 10⁹/L and greater than 10 x 10⁹/L should be confirmed by differential.

7. PROCEDURAL NOTES:

a. Specimen should be centrifuged within 20 minutes of insertion of float.

b. Centrifuged QBC tubes are stable for up to 4 hours prior to reading, provided they are stored vertically (closure end down) and away from heat and intense light.

c. Upon removing capillary tube from centrifuge, check the plasma meniscus. If it is above or below the red lines, discard the tube and prepare a fresh tube.

d. For an accurate platelet reading, sample must be read within 90 minutes of collection. Other parameters are stable for up to 4 hours.

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- e. A poorly mixed specimen will cause erroneous results in all parameters.
- f. Missing layers or skipping layers in the analysis will result in instrument flags of those results.

8. LIMITATIONS:

- a. Hematology parameters -- readings are valid only within the following parameters:
 - (1) Hematocrit: 25 - 55%.
 - (2) White Cell Count: $2.0 - 30.0 \times 10^9/L$.
 - (3) Granulocytes: 1 - 99%.
 - (4) Lymph/Mono: 1 - 99%.
 - (5) Platelet count: $80 - 600 \times 10^9/L$.
- b. Venous sample -- EDTA-anticoagulated blood not more than 4 hours old and at least 2/3 full for parameters other than platelets. For platelet counts, specimen must be centrifuged within 90 minutes.
- c. Reader must be placed on a flat surface counter with no vibration.
- d. Specifications.
 - (1) Operational Temperature: 20-32°C.
 - (2) Relative humidity: 10% to 95% non-condensing.
 - (3) Centrifuge: 120 Volts, 50/60 HZ, 4 Amps.
 - (4) Reader: 120 Volts, 60 Hz, 3 AG 1 1/2 Amp Slo-Blo.

9. REFERENCES:

- a. Brown, B.A., Hematology: Principles and Procedures. 6th ed., Philadelphia: Lea and Febiger, 1993.

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- b. "Assessment of Quantitative Buffy Coat Centrifugal Hematology Analysis in Primary Care, Emergency Care, Intensive Care and Blood Bank Settings", The Commission on World Standards of The World Association of Societies of Pathology, Las Vegas Nevada, November 2, 1985.