

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 November 01

MANUAL WHITE CELL COUNT BY UNOPETTE

1. PRINCIPLE:

Whole blood is diluted in acetic acid which hemolyzes the erythrocytes and preserves the leukocytes. The leukocytes are then counted in a standard hemacytometer.

2. SPECIMEN: Capillary or thoroughly mixed anticoagulated venous blood in EDTA tube, at least 3/4 full, is specimen of choice.

3. REAGENTS AND EQUIPMENT:

- a. Unopette Reservoir: Storage temperature 1-30°C. Protect from sunlight.
- b. Unopette capillary pipet.
- c. Hemacytometer and coverslip.
- d. Hand counter.
- e. Microscope.
- f. Petri dish.

4. QUALITY CONTROL:

- a. Technicians are trained and tested to proficiency; results are documented in their training files.
- b. On a regular basis proficiency counts are performed by the technician, results are reviewed by the supervisor, annotated in records, and appropriate action taken
- c. Verification of manual WBC count.

- (1) By QBC with a range of $\pm 1.0 \times 10^9$ WBC/L.
- (2) By differential slide WBC estimate with a range of $\pm 1.5 \times 10^9$ WBC/L.

5. PROCEDURE:

a. Prepare specimen.

- (1) Use the protective shield of the capillary pipet to puncture the diaphragm of the reservoir.
- (2) Fill capillary pipet with whole blood (pipet fills by capillary action). Wipe excess blood from outside of pipet.
- (3) Squeeze reservoir slightly to force out air.
- (4) Cover opening of overflow chamber of pipet with index finger and seat pipet securely in reservoir neck.
- (5) Release pressure on reservoir, then remove finger from pipet opening.
- (6) Squeeze reservoir gently two to three times to rinse capillary bore, forcing diluent up into, but not out of the overflow chamber, releasing pressure each time to return mixture to reservoir.

NOTE: If reservoir is squeezed too hard, the specimen may be expelled through the top of the overflow chamber, resulting in contamination of fingers.

- (7) Place protective shield over upper opening and gently invert several times to thoroughly mix blood with diluent.
- (8) Let stand for 10 minutes to allow red blood cells to hemolyze. Leukocyte count should then be performed within 3 hours.

b. Charge hemacytometer.

- (1) Convert Unopette to dropper assembly by withdrawing pipette from reservoir and reseal securely with capillary tube exposed.
- (2) Discard first 3 to 4 drops.
- (3) Carefully charge hemacytometer, by gently squeezing sides of reservoir until chamber is properly loaded.

- (4) Allow the cells to settle for 3 to 5 minutes before counting in a moist chamber.
- c. Counting the cells.
- (1) Use low power objective and low light.
 - (2) Performing the count.
 - (a) Count the WBCs in all 9 sq mm of the counting grid on both sides of the hemacytometer.
 - (b) The cells that touch the extreme lower and the extreme left lines are not counted.
 - (c) There should be no more than a 10-cell difference among the 9 squares counted.
 - (d) There should be no more than a 15-cell difference between the two sides.
 - (3) Average the two sides.

6. CALCULATIONS:

- a. Formula:

$$\text{Average \# of cells} \times \text{Dilution Factor} \times \text{Area Factor} \times \text{Depth Factor} \times 10^6 = \# \times 10^9 \text{ WBC/L}$$

OR

$$\text{Average \# of cells} \times \text{K Factor} \times 10^6 = \# \times 10^9 \text{ WBC/L.}$$

- b. Dilution Factor: 100.
- c. Area Factor: 0.111.
- d. Depth Factor: 10.
- e. K Factor: $111 = [\text{Dilution Factor (100)} \times \text{Area Factor (.111)} \times \text{Depth Factor (10)}]$.
- f. Example:

- (1) Chamber counts:

<u>Side #1</u>	<u>Side #2</u>
110 WBCs	114 WBCs

(2) Formula for average count:

$$\frac{\text{Side \#1} + \text{Side \#2}}{\text{Number of sides counted}} = \text{Average count}$$

(sides must be within 10% of each other)

$$\text{Side \#1 (110)} + \text{Side \#2 (114)} = 224.$$

$$224/2 = \text{Average count of 112.}$$

(3) Formula for Report:

$$\text{Average count} \times \text{K factor} \times 10^6 = \text{WBC count.}$$

$$112 \times 111 \times 10^6 = 12.432 \times 10^9 \text{ WBC/L.}$$

Report as 12.4×10^9 WBC/L.

7. RESULTS:

a. Normal Values

(1) Adult: $4.0 - 11.0 \times 10^9$ WBC/L.

(2) Newborn: $10.0 - 30.0 \times 10^9$ WBC/L.

(3) Results outside the normal values should be repeated.

b. CRITICAL LIMITS

(1) Adult: Less than 2.0×10^9 WBC/L or greater than 40.0×10^9 WBC/L.

(2) Children: Less than 2.1×10^9 WBC/L or greater than 42.9×10^9 WBC/L.

(3) Notify supervisor and doctor immediately; annotate notification on record.

8. PROCEDURAL NOTES:

a. Ensure venous specimen is thoroughly mixed and has sufficient volume to prevent dilution by anticoagulant.

b. When filling the capillary tube, ensure there are no bubbles and that the tube is completely filled.

- c. When wiping off the capillary pipet, do not wipe across tip.
- d. The hemacytometer should be placed in a petri dish containing damp gauze and 2 pieces of an applicator stick. Cover to prevent evaporation.
- e. Allow cells to settle in hemacytometer on flat surface with no vibration.
- f. A properly filled chamber will show square, straight edges. Over filling will cause a bulge or drop to form on the edge.

LIMITATIONS:

- a. Once diluted, count should be performed in 3 hours.
- b. Nucleated red blood cells (NRBCs) can alter results of white blood cell counts.
- c. Correct WBC count if more than 5 NRBCs are counted per 100 WBCs on differential.

10. REFERENCES:

- a. Brown, B.A., Hematology: Principles and Procedures. 6th ed., Philadelphia: Lea and Febiger, 1993.
- b. WBC Determination for Manual Methods, Unopette Microcollection System, Product Circulars 5804 and 5853, Becton-Dickinson, Rutherford, NJ 07070.