

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 DIFFERENTIAL
COUNT AND PLATELET ESTIMATE

1. PRINCIPLE:

The differential white cell count is performed to determine the relative number of each type of white cell present in the blood. This provides valuable information concerning infections and other disease processes. At the same time, a study of red cell, white cell, and platelet morphology is performed. Performing the differential smear after counting the cells allows the smear to be used as a double check of the white cell count and platelet count.

2. SPECIMEN:

Whole blood from a capillary puncture or EDTA tube at least 3/4 full.

3. REAGENTS AND EQUIPMENT:

- a. Microscope.
- b. Immersion oil.
- c. Differential cell counter.

4. QUALITY CONTROL:

- a. Slide.
 - (1) The RBCs should appear buff pink to orange.
 - (2) WBCs should have a blue nucleus with a lighter staining cytoplasm.

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- b. Technicians should be evaluated regularly on their ability to perform a differential. Results and appropriate actions taken should be documented.

5. PROCEDURE:

- a. Procedure for differential count.

- (1) Place the slide on the microscope stage with the smear side up and focus using the low power objective and low light.
- (2) Scan the blood smear, noting any unusual or irregular cells, or rouleaux formation.
- (3) Locate the portion of the smear where there are no overlapping of cells (thin area).
- (4) Place a drop of immersion oil on the slide.
- (5) Carefully change to the oil immersion objective (100x), focus and increase light intensity as needed.
- (6) Begin in the thin area of the smear.
- (7) Scan the slide in a figure "S."
- (8) Counts.
 - (a) White cell counts.
 - For WBC counts less than 20×10^9 WBC/L, count and classify 100 cells.
 - For WBC counts of 20 to 50×10^9 WBC/L, count and classify 300 cells.
 - If there is an abnormal percentage of cells in the differential count, classify 200 cells.

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- ✓ More lymphocytes than neutrophils (except in children).
- ✓ Over 11% monocytes.
- ✓ Over 10% eosinophils.
- ✓ Over 2% basophils.

(b) Nucleated red cells (NRBCs).

- Count # of NRBCs per 100 WBCs on a separate counter; report.
- Recalculate the WBC count if the NRBC count is greater than 6 NRBCs per 100 WBCs.

(9) Report any abnormalities of white cells.

b. Platelet estimate.

- (1) Scan the thin area, using the oil immersion lens.
- (2) Observe 10 fields, counting the platelets in each field, observing granulation and morphology.
- (3) Determine the average number of platelets observed per oil immersion field (OIF).
- (4) Report the platelet estimate and any abnormal morphology.

c. WBC estimate.

- (1) Scan thin area, using the 50x oil immersion lens.
- (2) Observe 10 fields, counting all WBCs in each field.
- (3) Average the number of WBCs seen per oil immersion field (OIF).
- (4) Multiply the number of WBC/OIF x 3,000 and compare with WBC count.
- (5) Document performance of WBC estimate. Report any discrepancies to supervisor.

d. RBC morphology.

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- (1) Scan thin area using the oil immersion lens.
 - (2) Observe 10 fields.
 - (3) Report RBC size and shape. Use the nucleus of a typical small lymphocyte as a comparison for normal size.
 - (4) Report any alterations in color, the amount of hemoglobin, or inclusions.
- e. Save all differential slides for 7 days.
6. RESULTS:
- a. Leukocyte differential -- normal values.
 - (1) Segmented neutrophils: 50% - 70%.
 - (2) Lymphocytes: 20% - 44%.
 - (3) Band neutrophils: 1% - 10%.
 - (4) Monocytes: 4% - 10%.
 - (5) Eosinophils: 0% - 4%.
 - (6) Basophils: 0% - 2%.
 - b. NRBCs reported as #NRBC/100 WBCs counted.
 - c. Platelets estimate.
 - (1) Adequate/normal -- 8 to 20 plt/OIF.
 - (2) Decreased --less than 8 plt/OIF.
 - (3) Increased -- more than 20 plt/OIF.
 - d. WBC estimate: $\pm 1.5 \times 10^9$ WBC/L of WBC count.

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e. RBC morphology.

(1) Normal findings.

(a) Normocytic -- normal cell size and shape.

(b) Normochromic -- normal hemoglobin content and coloration.

(2) Abnormal findings:

(a) Use specific terms.

(c) Grade degree of abnormalities.

- Slight: 1-5 cells/10 fields.

- Moderate: 6-15 cells/10 fields.

- Marked: Greater than 15 cells/10 fields.

7. PROCEDURAL NOTES:

a. RBC morphology.

(1) Anisocytosis -- variation in size of the cells.

(a) Macrocyte -- larger than normal.

(b) Microcyte -- smaller than normal.

(2) Poikilocytosis -- variation in shape of the cell.

(a) Drepanocyte (sickle-like cell).

(b) Ovalocyte (elliptocyte).

(c) Spherocyte.

(d) Dacrocyte (tear drop cell)

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- (e) Echinocyte (Burr cell).
- (f) Stomatocyte (cup cell).
- (g) Codocyte (target cell).
- (h) Schizocyte (red blood cell fragments).
- (i) Acanthocyte (irregularly spaced projections).
- (j) Crenated cells (mechanically produced, not reported).

(3) Alteration in color.

- (a) Hypochromasia -- increase central pallor.
- (b) Polychromasia -- diffuse basophilia.

(4) Inclusions.

- (a) Basophilic stippling.
- (b) Howell-Jolly bodies.
- (c) Cabot's rings
- (d) Malaria.

8. REFERENCE:

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