White Blood Cell tests (lab 6.2)

Background information and procedures for WBC counts

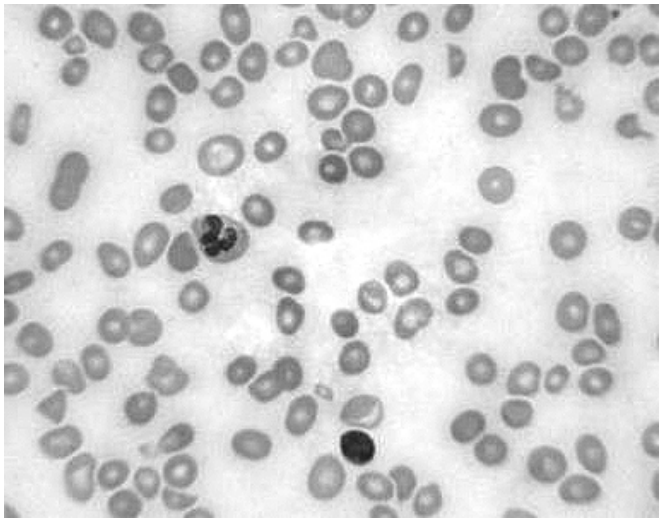
The background information for this lab are in lab 6.2 in your lab manual. We will, however, use different procedures.

a) Procedure for Differential White Blood Cell Count (procedure A)

1) Obtain a prepared slide with a blood sample. These slides have been stained with Wright's stain, which stains the WBC nuclei purple.

2) Turn the rheostat (the bulb voltage) to maximum but adjust the condenser iris diaphragm to as dim as possible. To perform the differential WBC count, you will need to use the 40X objective lens. But remember that it is almost impossible to focus this lens unless you first focus the 10X lens. Therefore, start with the 10X objective lens focused as close to the slide as possible. Then turn the course focus knob until the blood cells come into focus.
When you can see the blood cells using the 10X lens, switch to the 40X objective lens. You may need to adjust the light (use the condenser iris diaphragm) and fine tune the focus (use the fine focus knob only).

4) Most of the cells that are visible under the microscope will be RBCs, but there should be some WBCs also. Recall that RBCs do not have a nucleus but WBCs do. Find a region of the slide that has some WBCs, such as is shown below.



5) Identify all the WBC types in the field of view of your microscope. In the results section of this handout is a data table for the differential WBC count. Put one tally mark on the table in the row for each of the WBC types you identify in the field of view. .

6) After you have identified all the WBC type(s) in the field of view, move to a new field of view by turning the mechanical stage knobs. Repeat the identification and tallying in this new field of view, then move to another ffield of view. Keep moving to new fields of view until you have identified and tallied exactly 100 WBC types.

7) Using your tally, calculate for each WBC type its percent of the total WBCs. (The percents from all five types should add up to 100%).

8) Write the results in the results section of this handout. Show your results to your instructor before putting away your slide.

9) Compare your results with the normal percentages. Circle any percentages in your count that are outside the normal range. Next to the circle, draw an up arrow if your percent was above the normal range. Draw a down arrow if your percent was below the normal range.

10) Use the information in the "Clinical Applications" text box in lab 6.2 of your lab manual to list all possible diseases that the donor of the blood sample may have had.

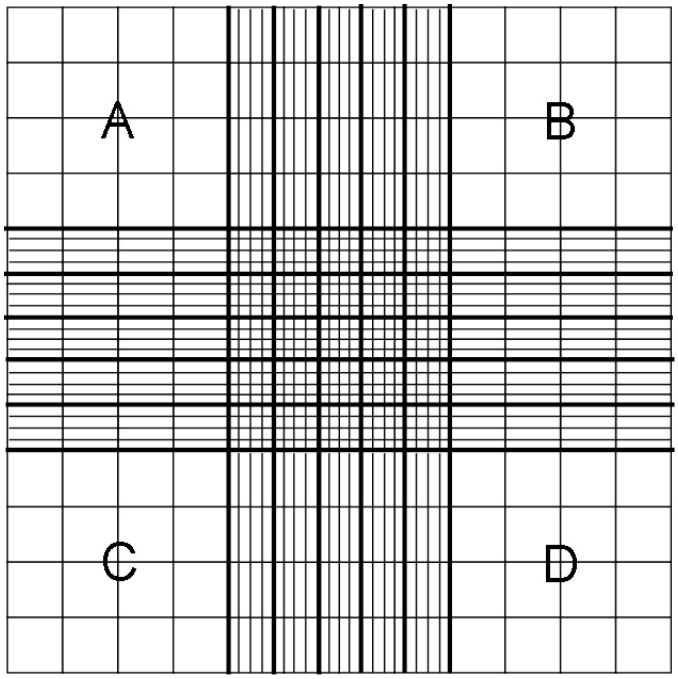
b) Procedure for Total White Blood Cell Count (procedure B)

1) Obtain a plastic box containing a hemocytometer (blood cell counting microscope slide) and a cover slip.

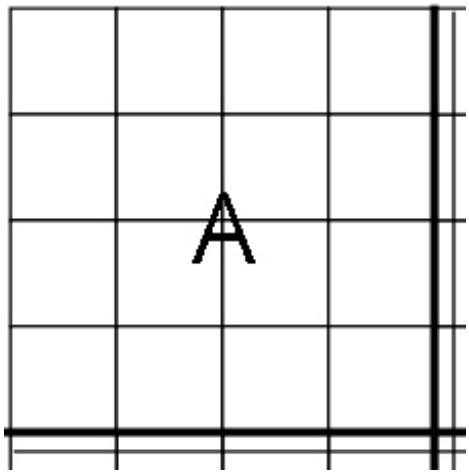
2) On the front desk are three test tubes (A, B, and C) containing synthetic blood diluted 1:20. Note that a special diluent was used that lyses (bursts) all the RBCs in these blood samples. This means that only WBCs are present in these samples. Using the dropper, place one drop on one of the silver regions of the hemocytometer. Then place the cover slip on top and let the cells settle for a few minutes.

3) Put the hemocytometer on your microscope stage. The counting grid is difficult to focus on unless you first adjust the light properly. Turn the rheostat (the bulb voltage) to maximum but adjust the condenser iris diaphragm to as dim as possible.

4) Start with the 4X objective lens above the silver region. The lens should be focused as close to the slide as possible. Locate the grid by turning the course focus knob slowly until the grid comes into focus. When it comes into focus, it should look like the one below (without the letters). If you are not able to see the grid, ask your instructor for assistance.



5) Move counting square A to the center of your field of view, then switch to the 10X objective lens. The square should now look like this (without the letter). Note that square A is made up of 16 single-lined smaller squares.



3) Count the WBSs in square A. Remember to include WBCs that touch the top and the left edges of the square, but don't include WBCs that touch the bottom or right edges of a square. Use a handheld clicker to help you keep count.

4) When you have counted all the WBCs in square A, use the mechanical stage knobs to move to square B. Count the WBCs in square B, then in squares C and D.

5) Add together the counts for squares A – D, then multiply the total by 50. The result is the total WBC count (the total number of WBCs per mm^3 of blood).

6) Write the results in the results section of this handout. Although each member of your lab group should count only one of the blood samples, be sure that your group counts all three samples. (You will need all three counts to fill in the results section and answer some of the review questions).

7) Show your instructor your group's results for Mr. A, Mr. B, and Mr. C before putting away the hemocytometers. When your results have been approved, wash the hemocytometer and the cover slip with water, then gently dry them with a paper towel.

c) Results section:

A) Differential WBC counts:

WBC type:	Counted percent	Normal percent range
Neutrophil	_____	55% - 75%
Eosinophil	_____	2% - 4%
Basophil	_____	0.5% - 1%
Monocyte	_____	3% - 8%
Lymphocyte	_____	20% - 40%

Diagnosis (list all possible diseases): _____

B) Total WBC counts:

Mr. A: _____ Mr. B: _____ Mr. C: _____
Diagnosis: _____ _____ _____

d) Review questions

1) Mr. X has a total WBC count of 7830. Mr. Y has a total WBC count of 3434. Mr. Z has a total WBC count of 12,672. Match one of these people to each of the descriptions below.

a) A patient with leukocytosis: _____ b) A patient with leukopenia: _____
c) A patient with leukemia: _____ d) A patient with AIDS: _____
e) A patient undergoing chemotherapy for cancer: _____
f) A patient with an autoimmune disease: _____
g) A patient with a typical viral infection: _____
h) A patient living under severe stress or depression: _____
i) A patient recovering from surgery: _____
j) A patient with a typical bacterial infection: _____
k) A normal patient: _____

2) Which leukocyte types would increase their percentage under the following conditions? Use the blue box in the differential count section of the lab manual to help answer these questions.

a) The patient has malaria: _____ b) The patient has smallpox: _____
c) The patient has diabetes: _____ d) The patient has asthma: _____
e) The patient has mononucleosis: _____