LAB OBJECTIVE
The student will be able to perform, within ≤20% accuracy as compared to the automated result, five manual white blood cell counts using the Unopette system.

PRINCIPLE
Free-flowing capillary or well-mixed anticoagulated venous blood is added to a diluent (ammonium oxalate) at a specific volume in the Unopette reservoir. The diluent lyses the erythrocytes but preserves leukocytes and platelets. The diluted blood is added to the hemacytometer chamber. Cells are allowed to settle for 10 minutes before leukocytes and platelets are counted. (Always refer to the manufacturer’s instructions for the procedure.)

SPECIMEN
EDTA-anticoagulated blood or capillary blood is preferred.

REAGENTS, SUPPLIES AND EQUIPMENT
Unopette reservoir no. 5854/5855 (1.98 ml of diluent + 0.02 ml blood = 1:100 dilution))
- Ammonium oxalate 11.45 g
- Sorensen’s phosphate buffer 1.0 g
- Thimerosal 0.1 g
- Purified water qs to 1000 mL
Unopette capillary pipet, 20-µL capacity
Hemacytometer and coverslip
Microscope
Lint-free wipe
Alcohol pads
Hand counter
Petri dish with moist filter paper
Neubauer hemacytometer

Hemacytometer side view

Note on the Hemacytometer
The hemacytometer counting chamber is used for cell counting. It is constructed so that the distance between the bottom of the coverslip and the surface of the counting area of the chamber is 0.1 mm.

The surface of the chamber contains two square ruled areas separated by an H-shaped moat. These two squares are identical, allowing the technologist to duplicate the cell count. Each has a total area of 9 mm$^2$ (1 mm on each side). These squares are divided into nine primary squares with an area of 1 mm$^2$. The four corner primary squares are used when counting leukocytes. *If the WBC is very low, you would count all 9 squares.* These 4 large corner squares contain 16 smaller secondary squares, each with an area of 0.04 mm$^2$. All 25 secondary squares of the center primary square are used to count platelets, and each of these 25 squares is further divided into 16 smaller tertiary squares (see figure).

The boundary lines of the central primary square are either double or triple. When the boundary line is double, all the cells within the square and those touching the innermost line are counted. If the boundary line is triple, all of the cells within the squares and those touching the middle line inward are counted.
Hemacytometers and coverslips should meet the specifications of the National Bureau of Standards and are so marked by the manufacturer. A standardized coverslip should be used that has been ground to fit the specifics of the hemacytometer, ensuring a uniform depth and therefore a constant volume. A regular coverslip cannot be used.

**QUALITY CONTROL**

A normal control specimen should be counted. Perform estimated WBC from Wright-stained peripheral smear to confirm result.

NOTE: For this lab exercise, QC will be precision check with automated WBC.

**PROCEDURE**

*Refer to figure for following instructions.*

1. Using the protective shield on the capillary pipette, puncture diaphragm of Unopette reservoir and add sample using a 20 μL capillary pipette provided with the Unopette system. *(A on illustration)*
2. Remove shield from pipette assembly by twisting.
3. Holding pipette almost horizontally, touch tip of pipet to blood. Pipet will fill by capillary action. *(B on illustration)* Filling will cease automatically when the blood reaches the end of the capillary bore in the neck of the pipet.
4. Wipe the outside of the capillary pipet to remove excess blood that would interfere with the dilution factor.
5. Squeeze reservoir slightly to force out some air while simultaneously maintaining pressure on reservoir.

6. Cover opening of overflow chamber of pipet with index finger and seat pipet securely in reservoir neck. (C on illustration)

7. Release pressure on reservoir. Then remove finger from pipet opening. At this time negative pressure will draw blood into reservoir.

8. Squeeze reservoir gently two or three times to rinse capillary bore forcing diluent up into overflow chamber, releasing pressure each time to return mixture to reservoir.

9. Place index finger over upper opening and gently invert several times to thoroughly mix blood with diluent. (D on illustration)

10. Let mixture stand 10 minutes before charging the hemacytometer.

11. To charge the hemacytometer, convert to dropper assembly by withdrawing pipet from reservoir and reseating securely in reverse position.

12. Invert reservoir and discard the first 3 or 4 drops of mixture.

13. Clean the hemacytometer and its coverslip with an alcohol pad and then dry with a wipe.

14. Carefully charge hemacytometer with diluted blood by gently squeezing sides of reservoir to expel contents until chamber is properly filled.

15. Place hemacytometer in moist Petri dish for 10 minutes to allow white cells to settle into the same plane. (Moistened filter paper retards evaporation of the plated specimen while standing.) Do not let the hemacytometer sit more than 15 minutes in the Petri dish because evaporation will begin to occur and cause erroneous counts.

16. Mount the hemacytometer on the microscope and lower its condenser.

17. Procedure for counting WBC’s
   a. Under 10x magnification, scan to ensure even distribution. Leukocytes are counted in all nine large squares of counting chamber.
   b. Count cells starting in the upper left large corner square. Move to the upper right corner square, bottom right corner square, bottom left corner square and end in the middle square.
EXERCISE 1: Manual Leukocyte Count  
MLAB 1315 Hematology

c. Count all cells that touch any of the upper and left lines, do not count any cell that touches a lower or right line.

Examples of white blood cells that are counted in a representative area.

Directions for counting:
- Count all cells that touch any of the upper and left lines, do not count any cell that touches a lower or right line.

18. Calculation for counting all 9 squares:
Total WBC/\text{mm}^3 = (\text{No. of cells} \times 10 \times 100)/9. Use the average \# of cells counted from both sides of the hemacytometer.

Note: When counting all 9 squares, one can more easily determine the total cell count by adding 10\% of count to total number of cells counted, and then multiplying this figure by 100 to get total leukocyte count. This is a simplification of the general formula described above which involves multiplying the cells counted by 10 to correct the depth of chamber and dividing by the number of squares enumerated.

Example
If an average of 60 cells is counted on both sides of chamber, add 10\% or 6, to 60 and multiply by 100 to get 6600 leukocytes/\text{mm}^3. This number can the be multiplied by a factor of 10^6 to derive SI units or 6.6 \times 10^9/L.
19. Calculation for counting less than 9 squares:

Example:
Average of cells: Total the cells in the four squares on each side and average the total.

Correction for dilution: The Unopette dilution is 1:100

# of squares counted: 4

Volume of square: One large square is 0.1 µL

# of cells in side 1 = 27
# of cells in side 2 = 29
Average # = 28

\[
\text{WBC/µL} = \frac{28 \times 100}{4 \times 0.1} = 7.0 \times 10^3/µL
\]

RECORDING YOUR RESULTS
Use the form provided to report your results. Record WBC’s counted in each square before proceeding to the next square.

Precision: The ability to reproduce a test result on the same sample. To determine if the two counts are in close enough agreement, use the following procedure:
1. Obtain the difference (C) between the Side A and Side B of the hemacytometer.
2. Calculate 2 S.D. as follows:
3. If C is less than 2 S.D., the results are acceptable. If greater, the counts must be repeated.

Example:
A = 430
B = 470
C = 40

C (40) is less than 2 S.D. (60) and therefore is acceptable.
Accuracy: The closeness of a test result to the true value. To determine if the two counts are accurate (within 20% of the automated count), use the following procedure:

Example:

Automated count = 7.0 x 10^9/L.
7.0 x 10^9/L - 20% = 5.6 x 10^9/L       Acceptable range is 5.6 - 8.4 x 10^9/L
7.0 x 10^9/L + 20% = 8.4 x 10^9/L

Manual count = 6.0 x 10^9/L so manual count is acceptable.

REPORTING RESULTS

Normal Values
Newborn 9.0-30.0 x 10^9/L
1 week 5.0-21.0 x 10^9/L
1 month 5.0-19.5 x 10^9/L
6-12 months 6.0-17.5 x 10^9/L
2 years 6.2-17.0 x 10^9/L
Child/adult 4.8-10.8 x 10^9/L

NOTES
1. Diluent and blood should be properly mixed before filling the hemactyometer.
2. The hemacytometer must be properly filled to avoid erroneous results in manual cell counting. If the chamber is overfilled, as indicated by the presence of excess fluid in the moat of hemacytometer, clean hemacytometer and recharge chamber. As with all manual counts, the diluent sample must be thoroughly mixed before charging the hemacytometer, which must be properly filled.
3. The chamber must be well-cleaned and free of debris which could be mistaken for leukocytes.
4. A highly elevated leukocyte count (leukocytosis) may make accurate counting difficult. In either instance, a secondary dilution should be made. When calculating the total count, adjust the formula to allow for secondary dilution.
5. There are physiologic variations to consider when performing WBC counts. Higher WBC counts (leukocytosis) are seen following exercise, emotional stress, anxiety, and food intake. Increased leukocyte counts are also seen in certain disease conditions such as bacterial infections, inflammation and leukemias. Decreased leukocyte counts (leukopenia) are seen in viral disorders, radiation induced leukopenia, chemotherapy induced leukopenia, aplastic anemia and megaloblastic anemia.
6. If more than 10 nucleated RBC’s are seen on the differential, the total leukocyte count should be corrected using the following calculation:

\[
\text{Corrected WBC} = \frac{\text{average total leukocyte count} \times 100}{100 + \# \text{ of NRBC’s/100 WBC’s on differential}}
\]

REFERENCES
**Manual WBC Report Form for Lab Exercise**

Student’s name: ____________________________ Date: ______________

Unopette Lot #: ____________________________ Expiration Date: _____________

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Side 1</th>
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Average: 

Difference between results: __________
Calculate 2 S.D.: 
Is the difference less than 2 S.D.? __________
Calculation of result:

Manual WBC result: ________________

Automated result: ________________
(Obtain from instructor)
Automated result range K<sup>20%</sup>: ________________

Is manual result within K<sup>20%</sup> of automated result? ________________
STUDY QUESTIONS

Name _______________________________
Date_________________________________

2 pts 1. Calculate the following white cell count if the dilution of the Unopette is 1:100. Show your calculations.

<table>
<thead>
<tr>
<th>Side #1</th>
<th>Side #2</th>
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<tbody>
<tr>
<td>Square 1 = 30 white cells</td>
<td>Square 1 = 27 white cells</td>
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<tr>
<td>Square 2 = 28 white cells</td>
<td>Square 2 = 27 white cells</td>
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<tr>
<td>Square 3 = 25 white cells</td>
<td>Square 3 = 24 white cells</td>
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<tr>
<td>Square 4 = 27 white cells</td>
<td>Square 4 = 24 white cells</td>
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2 pts 2. Calculate the following white cell count if the dilution of the Unopette is 1:100 and 9 squares were counted. Show your calculations.

Total cells counted on side 1 = 36
Total cells counted on side 2 = 32

2 pts 3. You are performing a manual white blood cell count. You have made the dilution and have filled the hemacytometer. A stat urinalysis is brought to the laboratory and you leave the hemacytometer sitting on the microscope stage while you perform the UA. Fifteen minutes later you return to complete the count. Should you count the dilution on the hemacytometer? Why or why not?
EXERCISE 1: Manual Leukocyte Count                                               MLAB 1315 Hematology

1 pt  4.  Why must the diluted blood sit in the Unopette for 10 minutes before it is plated on the hemacytometer?

1 pt  5.  Why must the loaded hemacytometer sit for 10 minutes before the manual WBC is read under the microscope?

2 pts 6.  You have performed a manual WBC and the counts from the two sides of the chamber are 232 and 268.  Are the counts close enough to accept? Show your calculations.

2 pts  7.  State normal WBC reference ranges for:

   Newborn _____________

   Child/Adult ____________

8 pts  8.  State four sources of error and indicate if the cell count would be falsely increased or decreased.

   A.
   B.
   C.
   D.

1 pt  9.  State the diluent that is used for unopette counts.

1 pt  10.  State the formula for correcting a WBC count if NRBCs are present.

2 pts 11.  Calculate the corrected WBC count for the following:

   Show your calculation.

   WBC count = 25,000/µL
# of NRBCs = 35/100 WBC

12. Define leukopenia and leukocytosis.

13. State 4 associated conditions for leukopenia.
   A. 
   B. 
   C. 
   D. 

14. State 2 disease (pathologic) conditions for leukocytosis.
   A. 
   B. 

15. State 4 physiologic conditions that can cause an elevation in the WBC.
   A. 
   B. 
   C. 
   D. 

16. How can the results of a manual WBC be confirmed?