MANUAL DIFFERENTIAL

LAB OBJECTIVE
1. To determine the relative number of each type of white cell present in the blood by performing differential cell counts on five relatively normal blood smears and five sets of abnormal blood smears within a ± 15% accuracy of the instructor's values.
2. To determine within one qualitative unit the red cell, white cell, and platelet morphology of each of the above blood smears.
3. To determine within ± 20% accuracy an estimate of the white cell counts and the platelet counts of each of the above blood smears.

PRINCIPLE
A stained smear is examined in order to determine the percentage of each type of leukocyte present and assess the erythrocyte and platelet morphology. Increases in any of the normal leukocyte types or the presence of immature leukocytes or erythrocytes in peripheral blood are important diagnostically in a wide variety of inflammatory disorders and leukemia. Erythrocyte abnormalities are clinically important in various anemias. Platelet size irregularities are suggestive of particular thrombocyte disorders.

SPECIMEN
Peripheral blood smear made from EDTA-anticoagulated blood. Smears should be made within 1 hour of blood collection from EDTA specimens stored at room temperature to avoid distortion of cell morphology. Unstained smears can be stored for indefinite periods in a dry environment, but stained smears gradually fade unless coverslipped.

REAGENTS, SUPPLIES AND EQUIPMENT
Manual cell counter designed for differential counts
Microscope, immersion oil and lens paper

QUALITY CONTROL
Training and experience in examining immature and abnormal cell morphology are essential. A set of reference slides with established parameters should be established to assess the competence of an individual to perform differential and morphological identification of leukocytes and erythrocytes. Participation in a quality assurance program continues to document the expertise of the hematologist in microscopy. Questionable or abnormal smears should be referred to a supervisor or pathologist for verification.

PROCEDURE
1. Focus the microscope on the 10X objective (low power). Scan the smear to check for cell distribution, clumping, and abnormal cells. In scanning the smear it is important to note
2. Examine the peripheral edge of the smear. If there is an increased number of white cells in this area, the differential count is inaccurate. Most of the cells at the edge of the smear are the large white cells, namely neutrophils and monocytes. This, therefore, shows poor distribution of white cell types and the smear is unacceptable.

3. If the smear is acceptable, estimate the white cell count by counting the number of WBC in each of 5 or 6 low power fields. Average the numbers. Multiply the average by 1000 and divide by 4. This number should be within ±20% of the actual white cell count. If it is not within this range, the white cell count and the estimation should be repeated.

\[
\text{(Average # WBC per 5 fields) \times 100} / 4
\]

4. Using the 100 X oil objective, place a drop of oil on the slide and examine the smear for platelets morphology and number. Find a thin area where red cells are not overlapping. In the appropriate area, count the number of platelets on about 4 to 5 successive fields. Average the number and multiply by 15,000 to obtain a rough estimate of the platelet count. A field with a normal platelet count would contain 9-26 platelets on 100X.

If platelets are clumped, this should be noted and one of the following comments added to the report:

1) Platelet count may not be accurate due to clumping. Platelets appear adequate.
2) Platelet count may not be accurate due to clumping. Platelets appear decreased.
3) Platelet count may not be accurate due to clumping. Platelets appear increased.
4) Platelet count may not be accurate due to clumping. Platelets appear too clumped for accurate estimate.

5. To perform the differential, choose the portion of the smear where there is close proximity but little overlapping of the red cells. They should have a central pallor. You should use the 100 X objective or 50X if available.

6. Begin the count in the thin area of the slide and gradually the slide as shown below.
LAB EXERCISE #10
MLAB 1315 Hematology

Count each white cell seen and record on a differential cell counter, until 100 white cells have been counted. If any nucleated red cells (NRBCs) are seen during the differential count, enumerate them on a separate counter. They are not to be included in the 100-cell differential count. They are reported as $\#\text{NRBC}/100$ WBCs and the WBC count must be corrected if there are $\geq 10$ NRBCs / 100 WBC. The following formula is used:

$$\frac{\text{WBC (in thousands) } \times 100}{\# \text{NRBC} + 100}$$

Example: $(14,200 \times 100)/ (100 + 21 \text{ NRBC}) = 11,800 \text{ WBC corrected}$

While counting the cells, make a note of any abnormalities present in the cells. *It is important to examine cellular morphology and to count leukocytes in areas that are neither too thick nor too thin.* In areas that are too thick, cellular details such as nuclear chromatin patterns are difficult to examine. In areas that are too thin, distortion of cells makes it risky to identify a cell type.

7. Results are expressed as a percentage of the total leukocytes counted. It is also helpful to know the actual number of each white cell type per µL of blood. This is referred to as the **absolute count** and is calculated as follows:

Absolute number of cells/µl = % of cell type in differential x white cell count

8. Examine the red cell morphology in a thin area of the slide where the red cells either do not overlap or lightly overlap. They should have a central pallor. Be conservative. In most cases an abnormality must be a consistent finding in order to be significant

1) Note any variations from normal and classify them as to:

- slightly, occasional or few
- 1+ = one or two cells are seen in every field
- 2+ = moderately increased but normal cells can still be found, 3-4/field
- 3+ = markedly increased in number; all but a few extreme cases of abnormality, >5/field
- 4+ = all cells are abnormal; reserved for extreme cases such as hereditary RBC abnormalities

*If no significant RBC morphology is seen, report RBC morphology as “Normal”.

2) Size: anisocytosis is a term that denotes variation in size. Grade as indicated above.

3) Shape: poikilocytosis is a term used to denote shape change. If this term is used, it should be clarified as the the particular shape variation, such as 2+ elliptocytes,
1+ schistocytes, etc. Review the terminology used to denote different shapes of RBCs such as target cells, tear drops, etc.

4) Inclusions: any RBC or WBC inclusions should be noted such as basophilic stippling, Howell-Jolly bodies, Dohle bodies, etc.

5) Any other abnormalities should be reported such as rouleaux, extracellular abnormalities such as certain parasites or suspected bacteria, etc.

REPORTING RESULTS
Reference values vary depending on age. For this exercise, the following values will be used (taken from Seton Medical Center):

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Birth</th>
<th>1 mo</th>
<th>6 yr</th>
<th>14 yr and ↑</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC x 10^9/µL</td>
<td>10-26</td>
<td>5-19.5</td>
<td>4.3-13.5</td>
<td>4.5-11.0</td>
</tr>
<tr>
<td>Neutrophils %</td>
<td>37-57</td>
<td>25-35</td>
<td>45-55</td>
<td>50-65</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>25-35</td>
<td>50-65</td>
<td>35-45</td>
<td>30-40</td>
</tr>
<tr>
<td>Monocyte %</td>
<td>3-9</td>
<td>2.5-7.5</td>
<td>0-8</td>
<td>0-10</td>
</tr>
<tr>
<td>Eosinophil %</td>
<td>1-3</td>
<td>1-4</td>
<td>1-4</td>
<td>0-4</td>
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<tr>
<td>Basophil %</td>
<td>0-1</td>
<td>0-1</td>
<td>0-1</td>
<td>0-1</td>
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</tbody>
</table>

PROCEDURE NOTES
1. A well-made and well-stained smear is essential to the accuracy of the differential count. The knowledge and ability of the cell morphologist is critical to high-quality results.

2. Before reporting significant abnormalities such as blasts, malaria or other significant finding on a patient’s differential, ask a more experienced tech to review the smear for confirmation. In clinical settings where a pathologist or hematologist is present, the smear is set aside for Pathologist Review.

3. If disrupted cells are present such as smudge cells or basket cells, not them on the report. It may be necessary to make an albumin smear to prevent the disruption of the cells. RBC morphology and WBC morphology must always be performed on the non-albumin smear.

4. When the WBC is very low (below 1,000/µL), it is difficult to find enough WBCs to perform a 100-cell differential. In this situation, a differential is usually performed by counting 50 cells. A notation on the report must be made that only 50 white cells were counted. Multiply each percentage x 2.

5. When the WBC is very high (>50,000/µL), a 200-cell diff may be performed to increase
the accuracy of the diff. The results are then divided by 2 and a note made on the report that 200 white cells were counted.

6. Never hesitate to ask questions concerning morphology or the identification of cells. The differential is one of the most difficult laboratory tests to learn. In fact, learning about cells and their morphology is a process that continues for as long as you perform differentials.

7. It is permissible to use a 50x objective to perform a differential, however keep the following points in mind: 1) If the WBC is increased, you should use the 100x to ensure that you will not skip cells in a field, 2) If you are having trouble identifying a cell, you must switch to the 100x in order to get a more detailed view.

REFERENCES
Seton Medical Center Hematology Procedure Manual
STUDY QUESTIONS

Name _________________________________

Date __________________________________

5 pt 1. List the normal adult ranges for each cell type.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Normal Range in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Segmented neutrophil</td>
<td></td>
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<tr>
<td>Lymphocyte</td>
<td></td>
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<tr>
<td>Monocyte</td>
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<tr>
<td>Eosinophil</td>
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<tr>
<td>Basophil</td>
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</table>

2 pt 2. Describe the procedure for estimating the white count.

2 pt 3. Describe the procedure for estimating the platelet count.
4. What is an absolute count on a differential?

5. Calculate the absolute counts for the following differential:
   Total WBC = 14.2 x 10^3/µL
   70% segs
   3% band
   20% lymphs
   3% monos
   4% eos

6. When performing a manual differential, you see 12 NRBCs. How do you report them?

7. Calculate the corrected WBC given the following results.
   WBC = 32.0 x 10^3/µL
   # NRBC/100 WBC = 20

8. Describe the proper area where you should begin your differential.

9. Draw a diagram of a blood smear and illustrate the path you would take in performing a manual differential.
2 pt 10. Upon scanning a stained peripheral smear, you notice clumped platelets. What would you do?

2 pt 11. Why do you avoid the thick area of the smear?

13 pt 12. Match the following:

A. microcytes
B. macrocytes
C. anisocytosis
D. hypochromia
E. polychromatophilia
F. spherocyte
G. poikilocytosis
H. schistocyte
I. Rouleaux
J. basophilic stippling
K. Pappenheimer bodies
L. Howell-Jolly bodies
M. Heinz bodies

1) Pale RBC with ↓ Hgb content
2) Single round (DNA) inclusion on RBC
3) Coinlike stacking of RBCs
4) RBC fragments
5) Variation in size of RBC
6) Aggregate of granules on RBC
7) variation in shape of RBC
8) RBC smaller than normal
9) RBC inclusions of denatured Hgb seen only with supravital stain
10) Small, round RBC with no central pallor
11) Bluish RBC
12) RBC larger than normal
13) Evenly distributed blue granules on RBC
Match the following:

<p>| | | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>A</td>
<td>toxic granulation</td>
<td>1) Neutrophil with &gt; 6 lobes in nucleus</td>
</tr>
<tr>
<td>B</td>
<td>Döhle bodies</td>
<td>2) Rod-like, needle shaped red inclusion in cytoplasm of myeloblasts and monoblasts</td>
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<tr>
<td>C</td>
<td>hypersegmentation</td>
<td>3) Nucleus condensed with no chromatin pattern; lobes often separate and round</td>
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<tr>
<td>D</td>
<td>auer rod</td>
<td>4) Heavy granulation in cytoplasm of neutrophils</td>
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<tr>
<td>E</td>
<td>smudge cell</td>
<td>5) Platelets aggregated around outside of a neutrophil</td>
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<tr>
<td>F</td>
<td>Pelger-Huet anomaly</td>
<td>6) Disintegrating nucleus of a ruptured lymph</td>
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<tr>
<td>G</td>
<td>platelet satellitism</td>
<td>7) Small light blue inclusion in cytoplasm of neutrophil</td>
</tr>
<tr>
<td>H</td>
<td>degenerating (pykrotic) neutrophil</td>
<td>8) Benign hereditary condition in which seg nuclei contain no more than 2 segments</td>
</tr>
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</table>
# ABNORMAL DIFFERENTIAL SCHEDULE

<table>
<thead>
<tr>
<th>Set #</th>
<th>Perform differential on these slides below:</th>
<th>On the slides below:</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1. Scan slide for abnormality</td>
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<tr>
<td></td>
<td></td>
<td>2. Show instructor</td>
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<td></td>
<td>3. Draw abnormality on report form</td>
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</tbody>
</table>
| 1     | A. Lymphocytosis  
B. Leukocytosis (post G-CSF therapy)  
C. Pelger Huet  
D. Normal if time allows | B. Toxic granulation  
E. Target cells, Howell-Jolly bodies |
| 2     | A. Hemoglobin C  
B. Sickle cell disease  
C. Eosinophilia  
D. Normal if time allows | E. Hemoglobin C crystals  
F. Sickle cells |
| 3     | A. Newborn baby  
B. CLL  
C. Normal if time allows | D. Rouleaux  
B. Smudge cells |
| 4     | A. Malignant lymphoma  
B. Hairy cell leukemia  
C. CMML | D. Pappenheimer bodies  
B. Hairy cells |
| 5     | A. Hemolytic anemia (↑ NRBCs)  
B. Blasts - ALL  
C. Blasts - AMML | D. Malaria  
E. Platelet clumps  
C. Auer rod |
Answer sheet for Student Differentials

Student Name:_________________________________

Date:_________________________________________

<table>
<thead>
<tr>
<th>Slide # or Name</th>
<th>Seg %</th>
<th>Band %</th>
<th>Lym %</th>
<th>Mono %</th>
<th>Eos %</th>
<th>Baso %</th>
<th>Other</th>
<th>WBC est</th>
<th>Plt est</th>
<th>RBC morph</th>
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