HEMATOLOGY SEQUENCE

BLOOD LABS

ORIENTATION — OBJECTIVES

Objectives

Procedure

1. Read the three sections that follow:
   a. Hematocrit
   b. Preparing a peripheral blood smear
   c. Microscopic Exam of a Peripheral Blood Smear (several parts)

2. An atlas of blood cells may be given out in class (you may keep it) or will be found in your textbook.

3. Please review the red and white cell morphology slides located on the web site and please review the powerpoint slide collection of Peripheral Blood Morphology imbedded in the heme course in Ctools and may also be found in the Hemepath website.

HEMATOCRIT

Principle

The hematocrit is the packed red cell volume that is expressed as a percentage of the volume of whole blood in a sample. The spun layers are seen as follows:
Micro-Hematocrit Method

Fill a heparinized micro capillary tube three-fourths full with well-mixed EDTA blood or fresh blood from a patient's finger (be sure to make a sufficiently deep puncture so that a free flow of blood is obtained; wipe away the first two drops). Seal one end of the tubes with Critoseal. Place the sealed tubes in the radial grooves of the micro-hematocrit centrifuge head with the sealed end away from the center. Make sure the inside lid is screwed on before closing the top lid and starting the machine. Set the timer for five minutes. When the centrifuge stops, read the hematocrits on the hematocrit reader. Instructions are printed on the reader. Be sure to note the buffy coat separately from the red cell layer if the buffy coat is larger than normal, i.e., 36/3 where 36 is the hematocrit and 3 is the buffy coat layer.
Preparing a Blood Smear (3 steps)

1. Place one drop of blood on the near end of one slide (Step 1, see diagram).

2. Back the edge of a second slide into the drop of blood at a 30 degree angle. The blood should run along the junction line of the two slides (Step 2, see diagram).

3. Push the second slide down the first creating a smear of blood on the first slide: push the full length of the first slide so that all of the blood is smeared out and ends in a "feathered edge."

Staining a Blood Smear (3 steps)

1. After the blood has dried, place the slide on staining corks in a staining boat (Petri dish). Flood with Wright's stain and let stand for two-three minutes. (This step fixes the cells because the Wright's stain is dissolved in methyl alcohol, a fixative.)

2. Add 20-30 drops of Wright's buffer (pH7) until a metallic sheen appears on the surface of the liquids, and mix the buffer and the stain by drawing them up and down in an eyedropper or disposable pipette. Let stand three minutes or longer.

3. Wash preparation with tap water and air dry.

If the above procedure produces a light stain, allow the staining procedure (after addition of buffer) to be in contact with the smear for a longer period of time. If the red cells are unduly orange, use a lesser proportion of buffer; if the red cells are dark or green, use a larger proportion of the buffer.
M I C R O S C O P I C E X A M O F A P E R I P H E R A L B L O O D S M E A R

IMPORTANT — Finding the Right Area on the Slide

A peripheral blood smear is made by the procedure described on the last page of this section (Preparing a Peripheral Blood Smear). It is a smear of blood on a 1x3” glass slide that looks something like this:

Most of the smeared area is not suitable for viewing at 100x (oil immersion) power, which is the magnification used for cytologic examinations such as the peripheral blood smear. In the unsuitable areas the red cells are piled on one another and the white cells appear small and dark because they are rounded (thus thick) and not flattened out. There is a small band on each slide just proximal to the feathered edge where the red cells are evenly-spaced, retain their central pale area, and the white cells are flattened out so that the microscope light transilluminates their nuclei. This is the only area on the slide suitable for the microscopic exam. Find it using the 10x (low power, non oil) lens, then place a drop of oil on the slide and swing the oil immersion objective on line.

Selection of Good Areas for Inspection

(adapted from Blood:Atlas and Sourcebook of Hematology by Carola T. Kapff and James H. Jandl, pp4-5)

Each peripheral smear should be examined first with a 10x objective lens to seek out fields where cells are evenly spread. By inspecting a "good" area, the viewer may estimate the proportion of red cells, leukocytes, and platelets and thus be prepared to recognize any important departure from the expected in other laboratory reports. With experience and adoption of a systematic scanning ritual, the observer can assess red cell and platelet morphology, as well as the more conspicuous aspects of leukocytes, during this low-power survey. It is good practice to ascertain initially the quality of the smear and to confirm with a 40x objective that most red cells have a normal, biconcave shape, before switching to the oil-immersion lens. An uncrowded, well-spread, and well-stained smear is shown in Figure 1 (X800). The red cells are light orange-pink and display normal areas of central pallor spanning half the cell diameter. In this field, the cells show slight variation in size (pokilocytosis) but no undue variation in shape (anisocytosis). The neutrophil is segmented. Evaluation of cytoplasmic granularity requires
inspection with an oil-immersion lens. The half-dozen, well-dispersed platelets seem normal. At center right, a platelet overlies a red cell. As platelets have a colorless, but structural, halo (clear zone) about them, their appearance when perched upon a red cell may mimic an inclusion body or an intracellular parasite. A platelet impressed on a red cell often displaces enough hemoglobin to obliterate the central pale area, as shown here.

At the periphery (edges) of blood smears made on coverslips or slides, there is a size-determined maldistribution of cell types, with an unrepresentative overabundance of the largest blood cells. Red cells deposited at the outer edge of the smear are deformed into shapes that can mislead viewers not adequately suspicious of peripheral areas. Red cells at smear margins display 3 conspicuous features: they appear flattened, cornered, and lacking in central pallor (Figure 2, X800). Wherever they abut they tend to have polygonal or faceted profiles. Flattened cells sometimes simulate spherocytes, but their largeness and normal color contradict this illusion. Crowding of several red cells into the band neutrophil at the bottom of the figure is characteristic of marginal artifacts as well as of smears that have partially dried before coverslip separation. If the entire smear resembles the scene depicted in Figure 2, the smear should be discarded and replaced by a different smear prepared from the same sample.

Figure 3 (X800) illustrates one of the common problems preventing evaluation of cell morphology. This smear was spread from an excessively large droplet of blood and is too thick with cells. In thick smears the red cells become superimposed, form extensive rouleaux, and shrivel during the protracted drying period. Thickly smeared cells may stain gray-brown, green, or blue. Furthermore, when red cells are amassed, aberrations in size and shape are concealed. The leukocytes and platelets also appear dark, primarily because of shrinkage during prolonged drying. The extent of deformation from shrinkage is attested to by the puckered neutrophil at the midright. Only with imagination can the shriveled cell at top be identified as a monocyte.

What Is The Microscopic Exam/How Is It Done?
The microscopic exam (done with the oil immersion 100x lens) has three distinct parts: (1) WBC differential, (2) RBC morphology, and (3) Platelet estimate. The sections below describe each of these components and provide the units and descriptors used in noting the results.

When a suitable area of the slide has been located (see above), place a drop of oil on the slide and swing the oil immersion (100x) objective into line. Using the mechanical stage, move back and forth within the "good area" using a zigzag pattern:

Separate the rows far enough so that you don't go over previously seen fields. Identify and keep count of each white cell you encounter until you reach 100 (%). Following completion of the WBC differential, review several fields to obtain a description of the RBC morphology. Then examine 10-15 fields to determine the platelet estimate.

**WBC Differential**

The white blood cell differential is a frequency distribution, expressed as percents, of all nucleated cells seen on a smear except nucleated red blood cells (NRBC). The WBC's that are expected in normal blood include polymorphonuclear neutrophils (PMN's or "polys"), band neutrophils, large and small lymphocytes, monocytes, eosinophils and basophils. However, any other cell present (abnormal, premature or whatever) is also counted and included in the 100% total of the differential. Broken cells (also called "smudge" or "basket" cells) can be included in the differential, or left out and described quantitatively, i.e., "many broken cells seen."

To count a WBC differential, make a vertical list of the names or abbreviations for cells that you expect to find, i.e. PMN (polymorphonuclear neutrophil), Bds (band neutrophils), M (monocyte), SL (small lymphocyte), LL (large lymphocyte), E (eosinophil) and B (basophil). Add to that list the names or abbreviations of cells not found in normal blood, but which you may find in the specimen you're examining. These latter cells, if present, are not to be treated as "skipocytes" because you didn't expect to see them. They are included in the 100% WBC differential as a description of that patient's white cell pathology. As you zigzag through the "good area" on the slide, keep count of the cells you identify using a "picket fence" method.
Example

<table>
<thead>
<tr>
<th></th>
<th>++++</th>
<th>++++</th>
<th>++++</th>
<th>++++</th>
<th>++++</th>
<th>++++</th>
<th>11</th>
<th>= 47%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bds</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3%</td>
</tr>
<tr>
<td>M</td>
<td>1111</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4%</td>
</tr>
<tr>
<td>SL</td>
<td>++++</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8%</td>
</tr>
<tr>
<td>LL</td>
<td>++++</td>
<td>++++</td>
<td>111</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13%</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2%</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1%</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>1111</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4%</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>111</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3%</td>
</tr>
<tr>
<td>Myeloblasts</td>
<td>++++</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6%</td>
</tr>
<tr>
<td>Unknown Cells*</td>
<td>++++</td>
<td>++++</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12%</td>
</tr>
</tbody>
</table>

*If you cannot identify a cell, don't skip or ignore it, but keep track of the number seen. In practice, you would then seek consultation from a more experienced morphologist and direct his/her attention to your problem cells. The probability is high that these cells will be directly related to the patient's diagnosis, and therefore shouldn't be disregarded just because you can't identify them. Your job is well done if you suspect something is wrong and press others to clarify the issue.

Nucleated red blood cells (NRBC) are counted separately and expressed as number seen/100 WBC counted. If more than 5 NRBC/100 WBC are present, the white blood cell count has to be corrected. If NRBC's are present, the white blood cell count determined by the Coulter Counter that provides cell counts is actually a total nucleated cell count (WBC and NRBC). The correction formula is:

\[
\frac{100 \times \text{Total cell count}}{100 + \text{NRBC}} = \text{Corrected white blood cell count}
\]

**RBC Morphology**

Using the oil immersion lens of the microscope, review several fields looking at the shape, size and configuration of the red blood cells. RBC morphology is recorded using the four classic descriptors listed below, i.e., Anisocytosis 2+, Poikilocytosis 1+, etc. If a slide does not show a descriptor, don't list the negative, i.e., it is not necessary to record No Anisocytosis, No Poikilocytosis, if these conditions are not present. After dealing with the four descriptors, record the presence of any specific RBC shapes seen with some estimate of their frequency (using words, not a plus or arrow system), i.e., "many sickle cells seen," or "occasional spherocyte seen," etc..

1. **Anisocytosis (variation in size)** - 1+ = just detectable
   4+ = macro and microcytes together

2. **Poikilocytosis (variation in shape)** -
   1+ = occasional abnormal cell
   4+ = 50% of cells abnormal

3. **Polychromatophilia (retained RNA)** -
   1+ = 1-2 blue cells per oil field
   4+ = 50% of cells blue

4. **Hypochromia (increased central pallor)** -
   1+ = just detectable
   4+ = faint rim of RBC left

**Platelet Estimation**
Platelets are always unevenly distributed on a Wrights-stained peripheral blood smear. Therefore, several fields (i.e., 10-15) need to be averaged to determine "number seen per oil field." The following approximate relationships exist.

<table>
<thead>
<tr>
<th>Average Number Platelets Per Oil Field</th>
<th>Approximate Corresponding Count</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 or less</td>
<td>&lt;50,000/μl</td>
<td>Markedly Decreased</td>
</tr>
<tr>
<td>4-8</td>
<td>50,000 - 200,000/μl</td>
<td>Moderately Decreased</td>
</tr>
<tr>
<td>9-20</td>
<td>200,000 - 400,000/μl</td>
<td>Normal</td>
</tr>
<tr>
<td>21-40</td>
<td>400,000 - 600,000/μl</td>
<td>Moderately Increased</td>
</tr>
<tr>
<td>40 + clumps</td>
<td>&gt;600,000/μl</td>
<td>Markedly Increased</td>
</tr>
</tbody>
</table>

Because assessing platelet number by viewing a peripheral blood smear using an oil immersion microscope objective (100x) is only an estimate, the recorded notation should not be a count but a designation that reflects the estimation process. The terms used in the "Interpretation" column above are one way to record the platelet estimate.

**Maturation Rules:** (Morphologic changes most cells go through as they mature from their "blast" form to their "mature" form.)

1. Cell size decreases
2. Nuclear size decreases
3. Nuclear/Cytoplasmic ratio decreases
4. Nucleolus is lost
5. Homogeneous chromatin develops a heterogeneous pattern characteristic of that cell line
6. Reddish purple chromatin changes to bluish purple
7. Deep blue cytoplasm becomes lighter
8. Empty cytoplasm develops a specific content characteristic of that cell line (hemoglobin, lysosomes, etc.)