BLOOD SMEAR PREPARATION

PRINCIPLE/SPECIMEN:
The well made peripheral blood smear is essential to the morphologic evaluation of hematologic disorders. Poorly made smears are misleading and may cause erroneous findings. EDTA whole blood or capillary blood may be used. Acceptable smears can be made up to 4 hours after drawing the EDTA whole blood specimen. Heparinized blood is unacceptable as it causes a blue background.

The ‘IDEAL SMEAR’ has a straight “feather” edge and becomes gradually thicker toward the point of origin (blood drop). The feather edge should include a large rainbow area with no ridges, tails, streaks or holes.

EQUIPMENT:
Clean 3 x 1" quality slides, untreated microhematocrit tubes, plastic pipets or wooden sticks, pencil

PROCEDURE:
1. Place a small drop of blood on a clean slide at one end. The drop of blood should be small enough so that the entire drop can be spread, but large enough so the smear goes 1/2 to 1 inch from the opposite end. The size of the blood drop will affect smear thickness and length.

2. Take a pusher slide and place it just in front of the drop of blood at a 30° angle from the smear slide. The angle of the pusher slide will affect smear thickness and length.

3. Draw the pusher slide back into the drop of blood and allow the blood to spread evenly. Gently push it forward with very little pressure and a moderate amount of speed across the surface of the smear slide. ●Use a new pusher slide edge for each smear made.

4. Slides must be properly labelled before staining with Wright's stain. Use pencil to label (first and last name) on frosted end. ■NEVER PRE-LABEL.

FOUR CRITERIA of a suitable blood smear by macroscopic observation:
1. The presence of a "squared" feathered edge (with rainbow area and no tails).
2. Proper thickness of blood film.
3. Proper length of the blood smear (2/3 to 3/4 of slide).
4. Proper width of the blood smear.

SPECIAL CONSIDERATIONS:
1. The EDTA tube must be mixed before obtaining an aliquot of blood. Tilt to mix, do not shake.

2. The blood drop should be spread immediately. The drop of blood should not rest on the slide for longer than 5 seconds before spreading or the bigger cells will be carried to slide edges which may distort the WBC differential result.

3. For normal bloods (normal # of RBCs), a 30° angle is best along with moderate speed.
   a. The smaller the angle of the pusher to the smear slide and the slower the "push" of the pusher slide, the thinner the smear. The larger the angle, the thicker the smear. ●INCREASE the angle of the pusher slide to make a shorter, thicker smear. ●DECREASE the angle of the pusher slide to make a longer, thinner smear.
   b. For blood with a low # of RBCs (anemia), increase the angle of the pusher slide. For blood with a high # of RBCs (polycythemia), decrease the angle of the pusher slide.

4. ●Do not apply pressure to the pusher slide as this will break cells and/or distort the distribution and morphology of the cells. The pusher slide should literally be glided across the smear slide.

5. Use clean slides only. Chipped slides cause tails and any blood on the pusher slide causes tails.
PRINCIPLE:
Wright’s stain is widely used for staining peripheral blood smears. Wright’s stain is a Romanowsky stain that contains Eosin and Methylene blue. Eosin (an acid dye) stains basic cell structures such as hemoglobin and eosinophil granules a red-orange color. Methylene blue (a basic dye) stains acid cell structures such as RNA in the nucleolus and cytoplasm, nuclear chromatin, and basophil granules a blue color. A combination of both dyes stains neutral cell structures such as neutrophil granules a pinkish-tan color.

Prepare and LABEL smears that meet the four criteria of a properly made blood smear.

STAINING PROCEDURE using QUICK Wright’s Stain:
1. Dip blood smears in **stain** for 15 seconds.
2. Dip blood smears in **jar 2** for 30-60 seconds.
   ■If jar 2 appears very blue, dump and refill with deaminized water.
3. Dip blood smears in **jar 3** for 10 seconds.
4. Wipe off stain on **backside** of smears. Let smears air dry in rack.

^^More than one slide can be stained at a time if placed back to back. Dip times are not exact.

Staining notes:
A properly stained smear looks pinkish-purple macroscopically; the red cells appear red-orange microscopically.

● For the Quick Wright’s stain, methanol in the stain fixes cells to the slide; deaminized water is the buffer. Staining does not occur until the buffer is added.

1. The RBCs may appear blue and the WBC nuclei deeply stained if:
   a. The staining time is too long.
   b. Washing is inadequate.
   c. The stain or buffer is excessively alkaline.
   d. The smear is too thick.
      ● Correct by decreasing stain time, decreasing pH of buffer, or making a thinner slide.

2. The RBCs may appear excessively red and the WBC nuclei poorly stained if:
   a. The staining time is inadequate.
   b. Washing of slide is excessive.
   c. The stain or buffer is too acidic.
      ● Correct by increasing stain time or increasing pH of the buffer.

3. Excess precipitate is caused by insufficient washing or inadequate filtering of the stain.