**Preparation of Blood Films**

**Principle:**
Blood film enables us to evaluate WBC, RBC, and PLT morphology, also, allows us to perform differential WBC count, furthermore estimation of WBC and platelets counts can be done on blood films. Blood films are made on glass microscopic slides.

**Sample:**
- Finger stick blood or EDTA anticoagulated venous whole blood may be used. Films of peripheral blood must be made immediately. Films may be made from EDTA anticoagulated blood as long as two to three hours after collection. All specimens should be free of clots.

**Procedure:**
1- Use clean standard size glass slides, wiped from dust just immediately before use.
2- Place a small drop of well mixed anticoagulated whole blood, in the center line of the slide, about 1.5 to 2 cm from one end, with the aid of a capillary tube.
3- Immediately, without delay, with the aid of a second clean slide with uniform smooth edges (spreader slide), with a 30–40 degrees angle, move back so blood drop will spread along the edge of the spreader slide, when this occurs, spread, or smear the film by a quick, forward motion of the spreader.
1. Place a small drop of whole blood on a VERY CLEAN slide. Hold a second slide at the angle shown.

2. While maintaining contact with the bottom slide, pull the top slide back to contact the drop, which will spread by capillary action.

3. Maintain firm contact with the bottom slide and push the top slide in one motion to produce the smear.

**Notes:**

- Before preparing the films, you must check that blood samples are free from clots, and this is done with two wooden applicator sticks. If clots are present the specimen is unsatisfactory.
- Films can be labeled with patient’s name and/or Lab. No. on the thick end of the film itself, after being dried, by using a pencil.

**DO NOT ATTEMPT TO CENTRIFUGE TO DISCARD PLASMA, THIS MAY DISTORT AND DISINTEGRATE THE CELLS, WHICH ARE OUR INTEREST!**
**Staining Blood Films**  
*With Romanovsky Stains*

Blood films are stained so that morphology of blood cells become more easily viewed, identified, and evaluated. In addition, blood films may be examined for the presence of blood parasites (Malaria, Trypanosoma, Babesia). Furthermore, stained blood films can provide important information about a patient's health, they may lead to a diagnosis or verify a diagnosis, or they may rule out a diagnosis.

Blood films are stained with one of the Romanovsky stains, which are universally used for staining blood films. Their remarkable property is creating distinctions in shades of staining granules differentially and this is dependent on two staining components: Azure B (the basic dye) and Eosin Y (the acidic dye). Other factors which affect the staining results include: 1) Staining time, 2) Ratio of Azure B to Eosin Y, 3) pH of the staining solution. Azure B will stain the acidic cell components (e.g. nucleus, because it contains nucleic acids; basophilic granules also take the Azure B staining because they contain heparin, which is acidic in origin), while Eosin Y will stain the alkaline basic components (e.g. Eosinophilic granules in eosinophils, because these granules contain spermine derivatives, which are basic in origin). Red cells have affinity for acidic Eosin Y dye, because it contains hemoglobin which is basic in origin.
**Romanovsky stains include:**
- Giemsa Stain
- Wright’s Stain
- Leishman Stain
- May-Grünwald Stain

**Leishman Stain Procedure:**
1- Let the films be air dried.
2- Put the films on a staining trough rack.
3- Flood the slides with the stain.
4- After 2 minutes (or more, if the stain is newly prepared), add double volume of water, and blow to mix the stain with water, until a shiny layer is seen.
5- After 5-7 minutes, wash with a stream of water.
6- Wipe the back of the slides with gauze.
7- Set the films in upright position on a filter paper to dry.
8- Read the blood films microscopically.

If delay in staining blood films may occur, fix the films in absolute methanol, for 1-2 minutes, but do not stain the slides until completely dried.
### Romanovsky Stain Blood Cell Characteristics

<table>
<thead>
<tr>
<th>No.</th>
<th>Cell Structure</th>
<th>Staining characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Red cells</td>
<td>Red or pinkish red</td>
</tr>
<tr>
<td>2</td>
<td>Nuclei of all cell types</td>
<td>Purple/violet</td>
</tr>
<tr>
<td>3</td>
<td>Lymphocyte cytoplasm</td>
<td>Blue</td>
</tr>
<tr>
<td>4</td>
<td>Monocyte cytoplasm</td>
<td>Grayish blue</td>
</tr>
<tr>
<td>5</td>
<td>Platelets cytoplasm</td>
<td>Light blue</td>
</tr>
<tr>
<td>6</td>
<td>Neutrophilic granules</td>
<td>Violet-pink</td>
</tr>
<tr>
<td>7</td>
<td>Eosinophilic granules</td>
<td>Orange-red</td>
</tr>
<tr>
<td>8</td>
<td>Basophilic granules</td>
<td>Purplish black/ Deep blue</td>
</tr>
<tr>
<td>9</td>
<td>Platelets granules</td>
<td>Purple</td>
</tr>
</tbody>
</table>

### Sources Of Errors In Staining

1- Stain Precipitate: May obscure cell details, and may cause confusion with inclusion bodies. Filter the stain before use.

2- pH of the buffer or water:

   - Too acidic pH causes too pinkish slides.
   - Too basic pH causes too bluish slides.

3- Improper stain timing may result in faded staining or altered colors:

   - Too long staining time causes too blue slides (overstaining).
   - Too short staining time causes too red slides.

4- Forced drying may alter color intensities and/or distort cell morphology.

5- Non-stain related errors:

1st-EDTA causes crenation of the cells after blood collection.
2nd- Severely anemic blood samples causes slower drying (before staining) due to excessive plasma.

3rd- Old blood specimens may cause disintegration in WBC’s and decrease in their numbers.

4th- Collection of blood in heparin causes blue staining of RBC’s with bluish background, which makes heparin unsatisfactory for routine hematology testing, also heparin induces platelet aggregation and clumping, with subsequent erroneous platelet count with automated counters.

Always filter the stain before each use, to eliminate stain precipitates.