PERIPHERAL BLOOD FILM PREPARATION

1. *Introduction*

Preparation of blood film is a fundamental technique in Hematology and blood collection is an important pre-analytical component. Red cells morphology and differential leukocyte count can be examined in peripheral blood film examination which is essential for hematology patients.

2. *General Component Required*

- Blood tubes with EDTA anticoagulant
- Clean glass slides
- Spreader with smooth edge
- Grease pencil
- Capillary tube
- Leishman's stain solution
- Distilled water or Sorensen's phosphate buffer (pH 6.8)

Figure 1 : Components of Blood Film Preparation

Note: It is necessary to follow a standard procedure for blood collection to get the most accurate and trustworthy results of peripheral blood film examination.

3. *Procedure*

a. *Peripheral Blood Film Preparation*

1) Gently invert the blood bottle for 5-7 times to mix thoroughly with the EDTA anticoagulant.
2) A small drop of blood is placed at one third of the slide with capillary tube.
3) The spreader or another clean slide is taken and placed it at an angle of 45 to the first slide.
4) The spreader is moved back until it meets the blood drop. When the blood has run along the edge of the spreader, the spreader is moved forward in a single rapid smooth movement pulling the blood after it.
5) The smear is allowed to dry in air and the blood film is ready for staining.

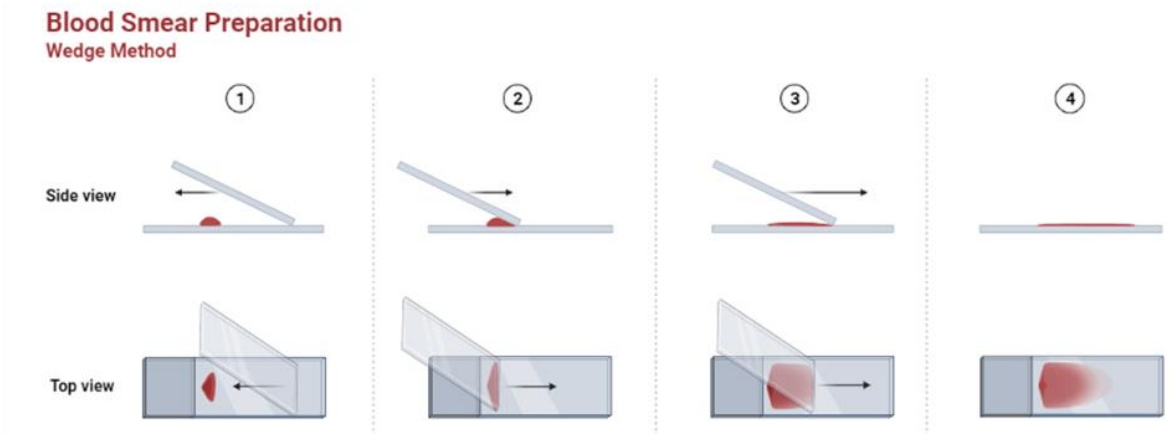

Figure 2 : Procedure of Blood Film Preparation

Note:

- *The faster the spreader slide is moved, the longer and thinner the film will be. The slower the slide is moved, the shorter and thicker the slide will be*
- *An angle greater than 30° makes the smear thicker; less than 30° the smear is thinner.*
- *A small drop of blood may be insufficient to prepare a slide of sufficient length, too large a drop may cause the smear to extend beyond the length of the slide.*

b. Blood Film Staining Method

1) Fix the blood film by applying 3minutes 70% alcohol solution
2) Dry the film carefully
3) Dye the film using:

- **Quick Giemsa stain**

4) Place the prepared blood film in undiluted Giemsa Stain for 1 minutes.
5) Rinse in deionized water for 2 minutes.
6) Allow the slide to dry on absorbent paper and clean the back of the slide.

- **Leishman's stain**

4) The prepared thin blood film is fixed in Leishman's stain for 2minutes.
5) Twice volume of Sorensen's phosphate buffer or distilled water is added to the stain.
6) The film is washed and differentiated with Sorensen's phosphate buffer or D/W until the blood show salmon pink color.
7) It is drained and dried in air at RT. The back of the slide is cleaned and examined microscopically.

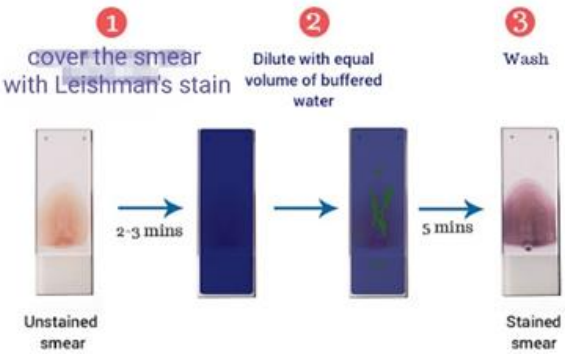

Figure 3 : Leishman Staining Method and Result Interpretation of Peripheral Blood Film

Note:

- *Stain artifact such as debris and precipitates may be caused by over-staining (excess stain contact time) and inadequate washing under running water.*
- *Weak staining can be occurred because of using fatigued (old) stains. Stains should be checked before using and make new stain if required.*

c. Blood film reading and result interpretation

1) Clean the back of the and place the smear under the microscope.
2) Put a drop of oil.
3) Set the microscope the read the smear with the X100 oil immersion objective.
4) Observe the blood elements per
    - Type;
    - Size;
    - Shape;
    - Coloration;
    - Inclusion.

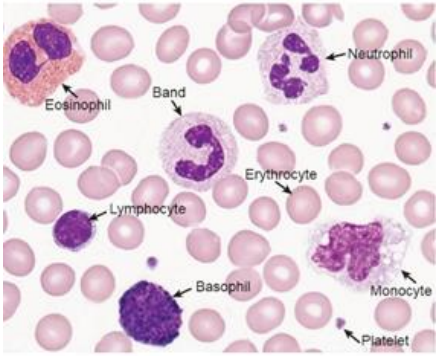

Figure 4 : Basic figure on result Interpretation on Peripheral Blood Film

Note:

- *For the white blood cell differentiation, 100 white blood cells element should be observed*
- *A percentage of each white blood cell identified are reported in %*
- *The absolute value is calculated with:* ***WBC element% x WBC total count***

4. Warnings and Precautions

- Pre-analytical variables that can affect the quality of film must be controlled. Samples are best analyzed within 2 hours of blood collection. Delay in preparation of blood smear may allow for the degeneration of the cellular elements of blood and may lead to false result interpretation.
- Do not use the expired EDTA blood bottles.
- Do not perform the test if the sample contain blood clots.
- Do not use the unclean slide and spreader.
- Wear personal protective equipment, such as gloves and lab coats when handling patient sample. Handle all specimens as if they contain infectious agents.
- Do not smoke, drink, or eat while handling specimen. Long hair is to be either tied back or up. Placing pens and pencils in the mouth are avoided.
- Any spills of any biological sample of reagent to be decontaminated and disposed to minimize the risk of spreading infectious agents to the other person.
- Before leaving the laboratory, make ensure that your work area is clean and tidy as instructed.
- Wash hands thoroughly after the tests are done.
- Dispose of all specimens and materials used to perform the test as bio-hazard waste.
- Laboratory chemical and biohazard wastes must be handled and discarded in accordance with all local, state, and national regulations.

References

- University of Medical Technology University: Hematology General Practical Guidance Handbook
- Dace and Lewis: Practical Hematology, Twelfth Edition
- http://www.blackwellpublishing.com/content/BPL_Images/Content_store/Sample_Chapter/1405142650/1405142650_4_001.pdf