Blood film

Spreading a drop of blood evenly across a clean grease-free slide using a smooth edged makes a smear or film of blood.

Preparation of blood film

1- Select a clean grease-free slide, never put fingers on the surface of the slide.

2- Gently touch a fresh drop of blood onto one end of the slide.

3- Using a beveled piece of glass a little narrower than the slide, allow the drop to spread along it (Fig.1).

4- Holding the slide and the "spreader" at a suitable angle, push the spreader along the slide, drawing the blood behind it, until the whole of the drop has been smeared and allow it to dry.

Staining of blood film

Before staining, the blood films need to be fixed with acetone free methyl alcohol for ¼ to 1 minute in order to prevent hemolysis when they come in contact with water while staining them.

White blood cells have structures that are acidophilic and basophilic structures, so they vary in their reaction pH. The nuclei are basophilic and stain blue. The highly basophilic granules also stain blue. Hemoglobin stains acidophilic or red.

Stain preparation and staining

1- Leishman's stain:

Powdered Leishman's stain 0.15 g

Aceton free methyl alcohol 137 ml

Mix and warm for 15 min. with shaking

2- Wright's stain:

Wright's stain powder 0.2 g

Aceton free methyl alcohol 100 ml

Let stand this solution for a few days.

Procedure:

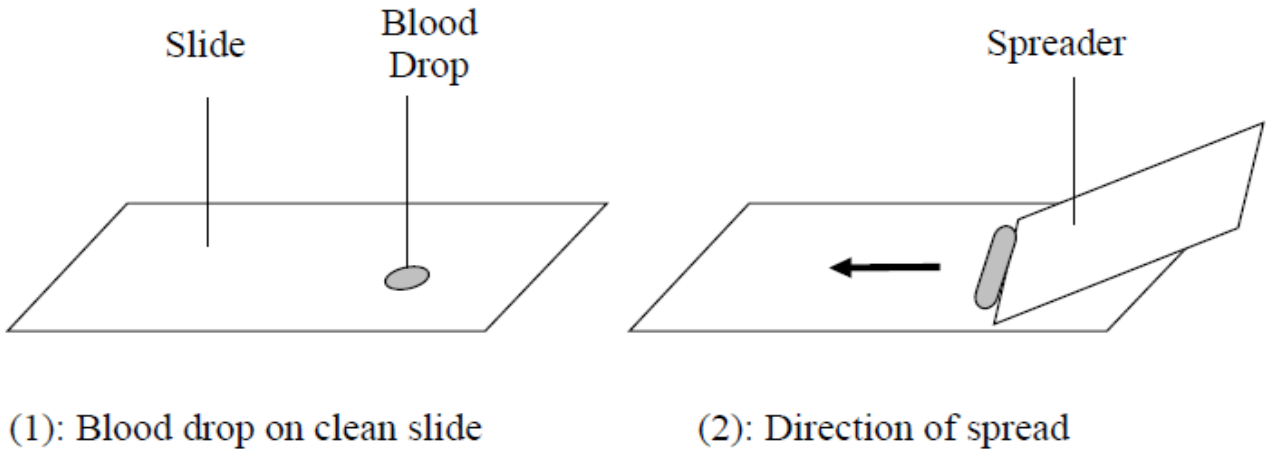
1- Cover the blood film with 8-10 drops of Leishman's stain for 1-2 min.

2- Dilute the stain with an equal volume of distilled water (DW) and mix by gentle rocking then leave it for 10 min.

3- Wash with (DW).

4- Drain and dry in the air at room temperature.

5- Clean the back of the slide and examine it microscopically.



(Figure 1 blood smear preparation)

Notes on technique

The slide should be very clean and free from any greasy materials to get a good spreading. The film should be such that there is some overlap of the red blood cells, diminishing the separation near the tail of film, but it should not be so thick that the leukocytes in the body of the film are badly distributed, if films are made too thinly, or if a rough edged spreader is used. Many of the leukocytes perhaps 50% of them accumulate at the edges in tail.

A well spread blood film should have the following characteristics (Fig.2):

1. Lateral edges 2. An adequate zone of morphology 3. Straight feature-edge.

4. Adequate length.

The zone of morphology is the area of the film where the RBCs barely touch each other (Fig.3), this is appropriate area for carrying out blood film examination.

Preparing a good quality smear depends on three main factors:

1- The size of the drop of blood.

2- The angle applied to spreader.

3. The speed and steadiness in pushing the spreader.

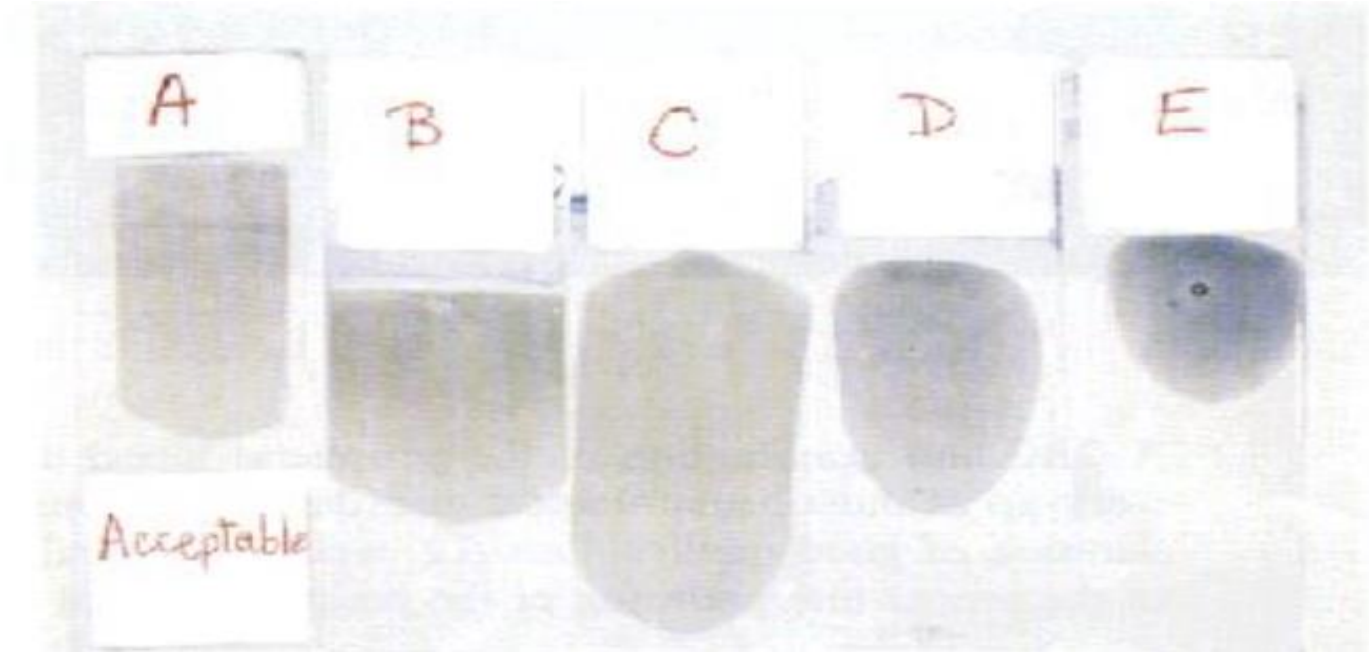
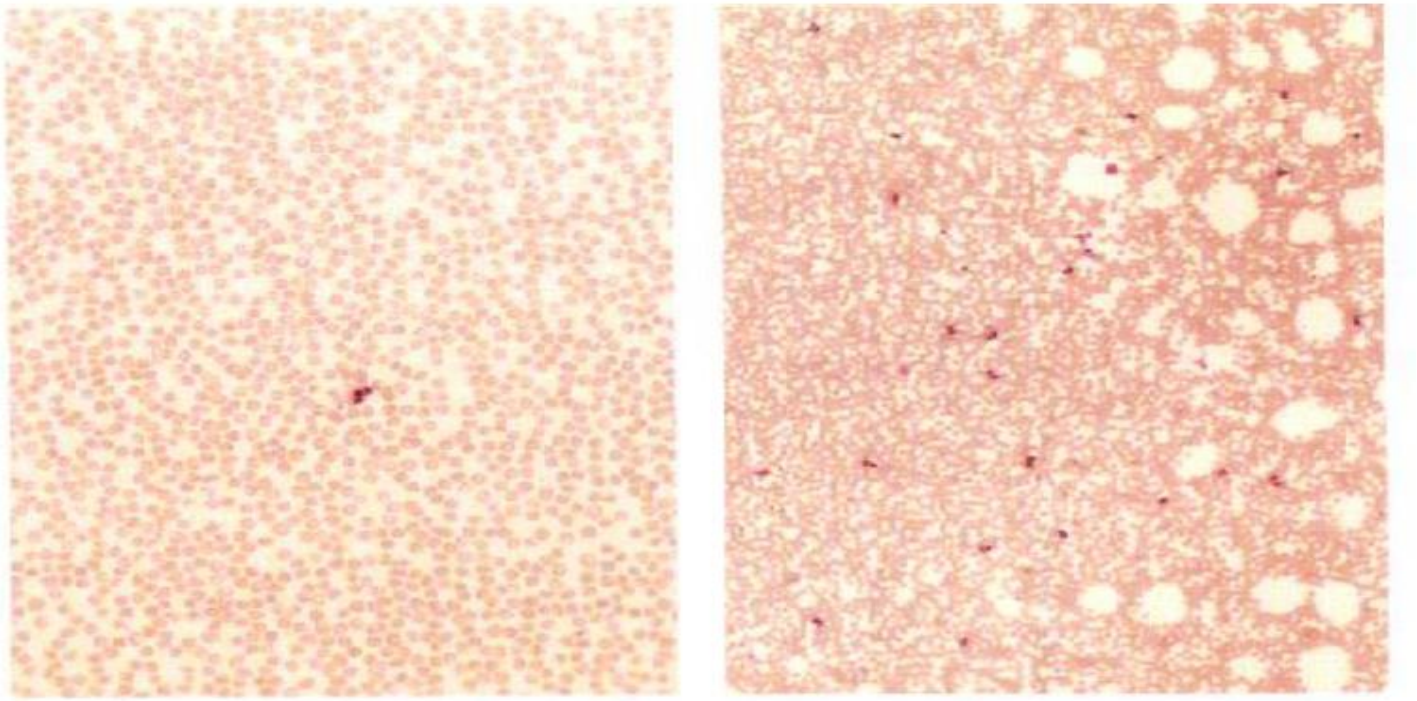


Figure 2 Examples of blood films. The optimal blood film **(A**) is thin and of medium length. The others are suboptimal because of the lack of lateral edges **(B, C)**. A curved zone of morphology **(D)**. Or excessive thickness **(E)**.



A B

Figure (3): a: Zone of morphology. The RBCs are well separated from each other. **b:** Thick film with no zone of morphology (RBCc are crowded).

References:

1- Hoffbrand, A. V., & Steensma, D. P. (2019). Hoffbrand's essential haematology. John Wiley & Sons.
2- Adewoyin, A. S. (2014). Peripheral blood film-a review. *Annals of Ibadan postgraduate medicine*, *12*(2), 71-79.
3- Neel, J. A. (2013). Blood smear basics. *NC State college of Veterinary Medicine. North Carolina: Raleigh*.