Al-Mustaqbal University
College of Science
Department Medical Biotechnology
Animal Physiology\ Stage 2
Practical Lab. 2
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Homological test

Blood Smear Preparation

There are multiple methods to prepare a blood smear. Each person may have their own preference/methods for success. When first learning, try multiple methods to determine which process works best for you. Blood smears are made using fresh blood or EDTA blood.

Three basic steps to make blood film:-

1. Preparation of blood smear.

2. Fixation of blood smear.

3. Staining of blood smear

WEDGE BLOOD SMEAR

Specimen:-

- Peripheral blood smear made from EDTA-anticoagulated blood.

- Smears should be made within 1 hour of blood collection from EDTA specimens stored at room temperature to avoid distortion of cell morphology.

- Blood smears can also be made from finger stick blood directly onto slide.

Equipment:-

- Spreaders.
- Clean slides.

- Blood capillary tube or micropipette 10 µL .

Procedure:-

1. Fill a capillary tube three-quarter full with the anticoagulated specimen.

2. Place a drop of blood, about 2 mm in diameter approximately an inch from the frosted area of the slide.

3. Place the slide on a flat surface, and hold the narrow side of the non frosted edge between your left thumb and forefinger.

4. With your right hand, place the smooth clean edge of a second (spreader) slide on the specimen slide, just in front of the blood drop.

5. Allow the blood to spread almost to the edges of the slide.

6. Push the spread forward with one light, smooth, and fluid motion. A thin film of blood in the shape of a bullet with a feathered edge will remain on the slide.

7. Label the frosted edge with patient name, ID# and date.

8. Allow the blood film to air-dry completely before staining. (Do not blow to dry. The moisture from your breath will cause RBC artifact).

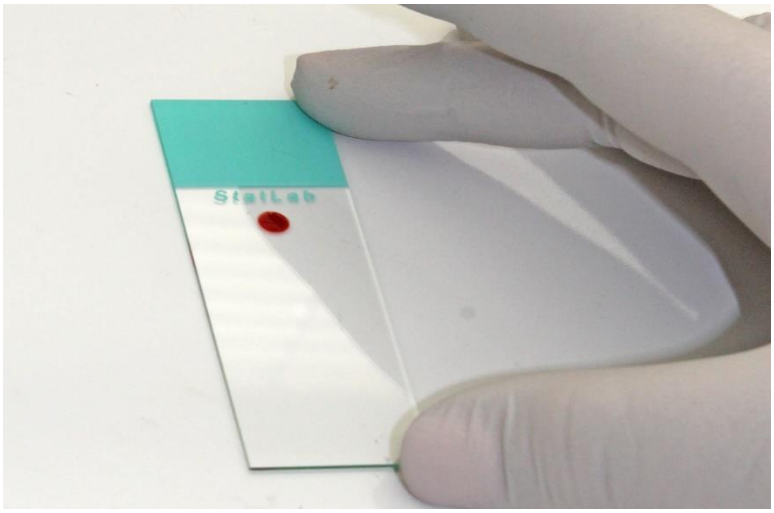
9. Hold the spreader slide at a 30° angle, and draw it back against the drop of blood The shape of blood film.

Step 1

Using lens paper, gently wipe two glass slides to remove any dust or glass fragments. Place the glass slides on an even surface.

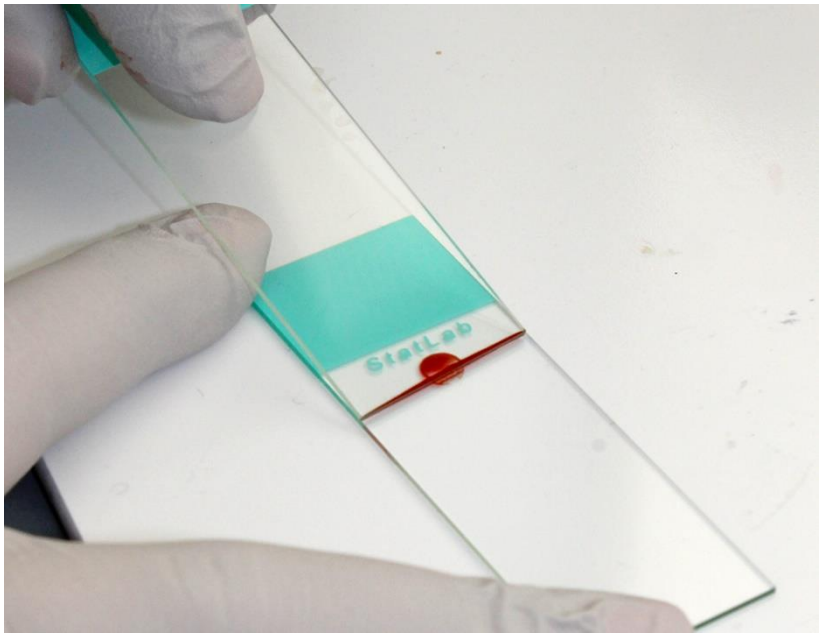
Step 2

Mix blood thoroughly (if not a fresh sample). Place a small drop of blood on one end of one glass slide. Hold the top and bottom edges of the slide with the thumb of your non-dominant hand.



Step 3

Using your dominant hand, place the edge of the other slide at an approximately 35-45^{o} angle on the first glass slide, in front of the blood drop. Using gentle pressure, gently pull the second slide back into the blood drop and allow the blood to spread to the edge of the slide.

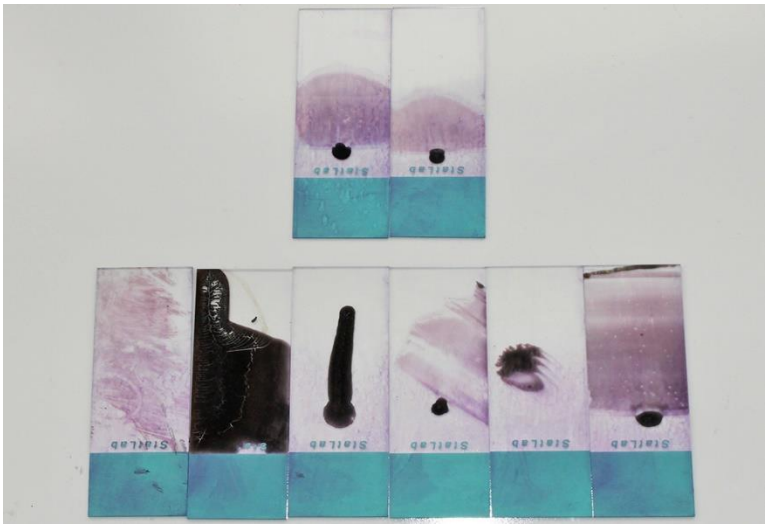


Step 4

To spread the blood, rapidly but gently push the top slide forward through the remainder of the slide. It is important to keep gentle, equal pressure throughout the whole process, and do not lift the top slide before it reaches the edge of the bottom slide. A feathered edge should be present.

The top two slides are examples of proper blood smears.

The bottom row displays poorly prepared blood smears.



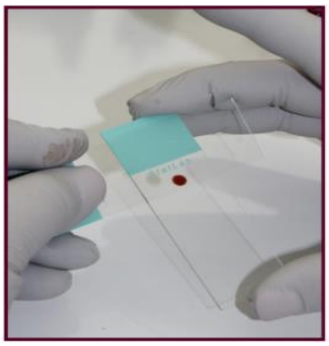
Step 5

After preparation, the smear should be labeled and dried (air dryer or waving method).

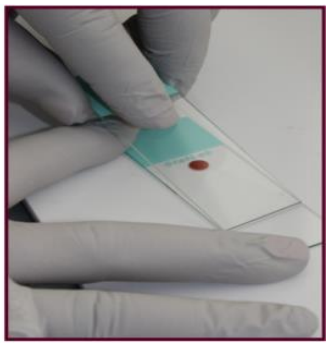
Sending Blood Smears

Blood smears can be examined by trained veterinary technicians, veterinarians, or sent to a diagnostic laboratory with whole blood. When sending blood smears, ensure they are protected from formalin and cold packs. Blood smears should not be refrigerated since warming to room temperature can cause condensation and cell lysis.

Pull vs Push



Holding vs Counter



Troubleshooting

Problem	**Solution**
Too thick or too short	Try decreasing the angle of the spreader slide or decreasing the size of the initial blood drop.
Too thin or too long	Try increasing the angle of the spreader slide or decreasing the size of the initial blood drop.
Streaking	Try cleaning the edge of the spreader slide.

Characteristics of A Good Smear:-

1. A good blood film preparation will be thick at the drop end and thin at the opposite end.
2. The blood smear should occupy the central portion of the slide.
3. The blood smear should not touch the edges. except for point of application.
4. Should be margin free.

The thickness of the spread when pulling

Is determined by:

1. The angle of the spreader slide. (the greater the angle, the thicker and shorter the smear).

2. Size of the blood drop.

3. Speed of spreading.

Common causes of a poor blood smear

1. Drop of blood too large or too small.

2. Spreader slide pushed across the slide in a jerky manner.

3. Failure to keep the entire edge of the spreader slide against the slide while making the smear.

4. Failure to keep the spreader slide at a 30° angle with the slide

Determination of packed cell volume(P.C.V.) Hematocrit

1-Hematocrit is defined The percentage by volume of packed red blood cells in a given sample of blood after centrifugation.

• The hematocrit may also be referred to as Packed Cell Volume (PCV) or erythrocyte volume fraction (EVF).

Purpose: This is a benefit test for diagnosis of certain blood disorders for example. Increased P.C.V values indicate polycythemia, Diabetes mellites, etc while Decreased P.C.V values indicate anemia, Leukemia and Hypothyroidism, etc

P.C.V. test:

1. Material required:
1-Blood sample.
2-Microhaematocrite centrifuge.
3-Microheaematocrite reader.
4- Wax or paste.
5-Cotton, alcohol.
6-Lancet.
7-Capillary tube.

2. Procedure:

1. Clean your finger with 70% alcohol and let dry.

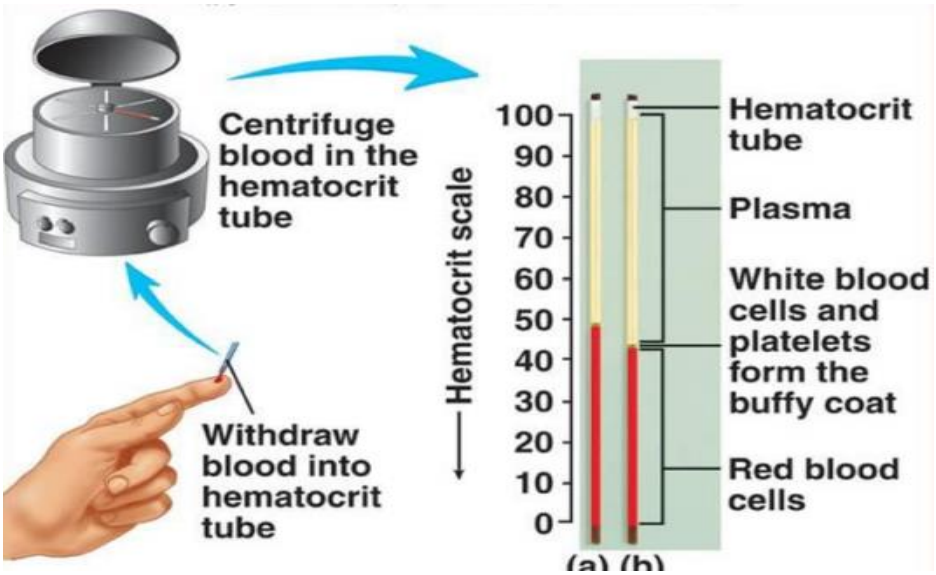
2. Prick finger with lancet, near the tip but not too close to the nail. Prick so that blood flows freely. Try squeezing up from your wrist if blood does not flow after pricking finger.

3. Place the tip of a capillary tube onto a drop of blood on your finger.

4. Call your instructor to seal the tube.

5. The instructor will spin the tubes in a centrifuge (5 minutes at 10000 rpm),.

6. Using a special reading device(since the capilary tube is not graduated).

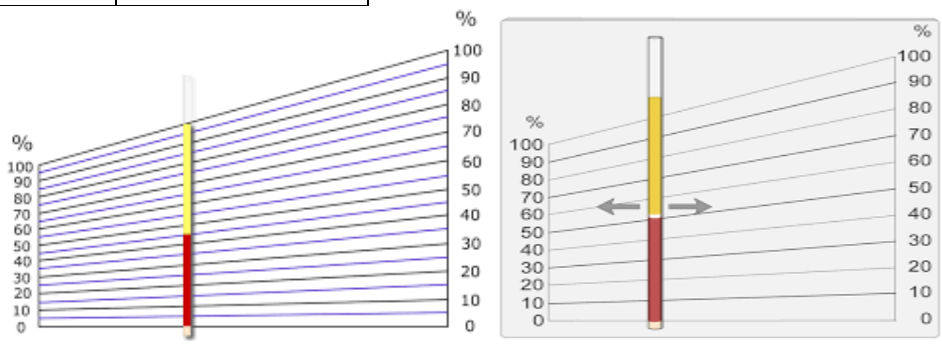


The method of calculating the value of the PCV own ruler:

The lower end of the capillary tube is placed at the zero line on the ruler from the left and then moves to the right until the top line of the plasma(the transparent part of the fluid in the tube)intersects with any line on the ruler. The value along the line between the red and transparent lines in the tube This reading is the value of p.c.v.

The normal value is as follows:

Man	40-54%
Women	37-47%
Newborn	55-58%



PCV is affected by the shape, & the number of the RBCs & the plasma volume.

• **High PCV either indicates either increase in number of circulating RBCs or decrease** in plasma volume seen in cholera due to loss of water in the stool

• A low PCV indicates either decrease in RBC or increase in plasma volume.

A lower than normal hematocrit may indicate:

• An insufficient supply of healthy red blood cells (anemia)

• A large number of white blood cells — usually a very small portion of your blood — due to long-term illness, infection, leukemia, lymphoma or other disorders of white blood cells.

• Acute kidneydisease (lower Erythropoietin production lead to less RBCs production by the bone marrow).

• Pregnancy may lead to women having additional fluid in blood. This could potentially lead to a small drop in hematocrit levels

A higher than normal hematocrit may indicate:

• Abnormal increase in red blood cells (erythrocytosis)

• A disorder, such as polycythemia vera that causes your body to produce too many red blood cells (in polycythemia it may rise to as high as 70 %).

• At higher altitudes, there is a lower oxygen supply in the air and thus hematocrit levels may increase over time.

• Low blood oxygen levels (hypoxia)

• Lung or heart disease — if the body senses low oxygen levels, it will make more red blood cells in an effort to increase the amount of oxygen in the blood

• Dehydration.

• Burn(due to loss plasma).