

EXERCISE 10: PREPARATION OF PERIPHERAL BLOOD SMEARS

Skills: 10 points

Objectives:

1. Perform a capillary puncture using aseptic technique.
2. State the two samples which may be used to prepare a blood smear.
3. Prepares an acceptable blood smear for routine Hematological studies using proper blood drop size, correct angle of spreader slide, and adequate speed.
4. Describe how a “differential” is performed.
5. State three factors which may be altered slightly to produce an acceptable blood smear.
6. State how blood viscosity will affect the angle of the spreader slide.
7. List three qualities of the appearance of the “perfect” blood smear.
8. List three precautions to follow when preparing blood smears.
9. List five causes of poor blood smear preparation.
10. Demonstrate the appropriate technique for preparing a peripheral blood smear

Discussion

Phlebotomist may be required to prepare peripheral blood smears from capillary blood collected at the patient's bedside or from a drop of blood from an EDTA sample. Blood smears are made by placing a well mixed drop of blood 1 to 2 mm in diameter 1/4 inch from the frosted edge of the slide. The drop should be in the center line approximately. The aim is to get a region where the cells are spaced far enough apart to be counted and differentiated. The blood smear is then fixed and stained for microscopic examination.

Blood smears are needed for microscopic examination of the blood. They may be prepared from venous blood or from capillary blood. The most common blood smear is used for the *differential*. The differential involves the Technician or Technologist will count 100 white blood cells (WBCS) noting the number, type and characteristics of blood cells. This aids the physician in diagnosing viral infections, bacterial infections and certain blood disorders such as leukemia. Another procedure, thick smears, are used to detect malarial parasites in the blood.

Procedure

It takes considerable practice to consistently make perfect blood smears. The handmade wedge or thin slide is the most commonly prepared blood film.

Three factors may be altered slightly to produce a perfect blood smear: *speed, angle and drop size*.

1. 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.
2. An angle greater than 30° makes the smear thicker; less than 30° the smear is thinner.
3. 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.

After preparing the blood smear it is labeled with the patient's name, identification number and date.

Blood Viscosity and Angle of Spreader Slide

Blood viscosity (thickness of blood) will vary from patient to patient. Two main conditions will affect the viscosity of the blood. Any condition which causes an increase in the hematocrit, such as polycythemia (too many red blood cells) will increase the viscosity of the blood. When the viscosity is increased, a thinner slide is needed. To accomplish this, decrease the angle between the spreader and the slide. Any condition which will cause a decreased hematocrit, such as anemia (abnormally low number of red blood cells) will cause a decrease in the viscosity. When the viscosity is abnormally low, a thicker slide is in order. To make thicker slides, increase the angle between the spreader and the slides.

Appearance of an Acceptable Blood Smear

The perfect blood smear *has a feathered edge that is nearly square, has a rainbow sheen when reflecting the light and is exactly one cell thick in the feathered edge when viewed microscopically.* Proper preparation of the blood smear is critical for obtaining accurate results on the differential.

Precautions

1. Make the blood smear without delay. As soon as the drop of blood is placed on the glass slide, the smear should be made without delay. Any delay results in an abnormal distribution of the white blood cells, with many of the large white cells accumulating at the thin edge of the smear.
2. The spreader slide can only be used ONCE to prevent carry over of patient's blood on to the next blood smear.
3. Position your fingers on the spreader slide as far down as possible and apply even, moderate pressure to the spreader slide. If the fingers are too high up on the spreader slide excess pressure will cause the slide to break resulting in a cut. Any pressure exerted on the spreader slide should be directed across the slide in the direction that the film is made rather than down on the stationary slide.

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.
5. Failure to push the spreader slide completely across the slide.

VISIT: http://www.funsci.com/fun3_en/blood/blood.htm to see pictures of the different types of white blood cells which are counted when performing a differential.

Procedure: Preparation of Peripheral Blood Smears

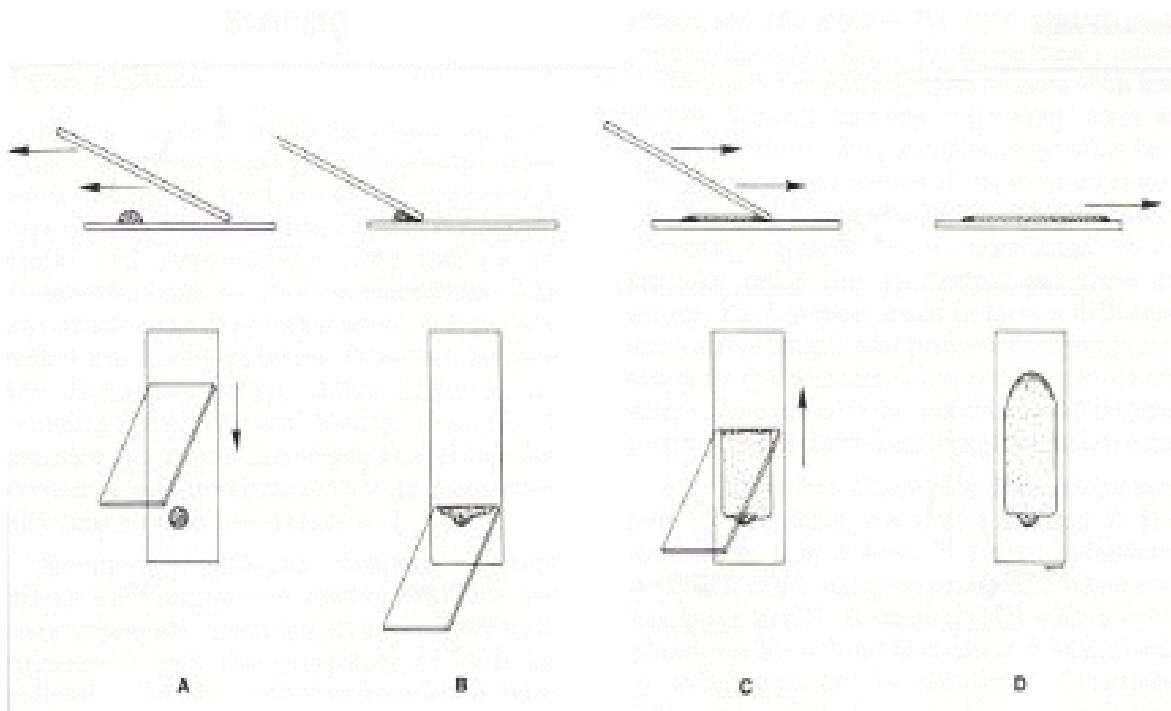
Materials:

1. Blood filled capillary tube **OR** purple top with diff-safe dispenser.
2. Lens cleaner
3. Lens paper
4. Glass microscope slides.
5. Biowipes
6. Gloves

Instructions:

1. Select two glass slides that are CLEAN and free of chipped edges. Fingerprints, grease, dust, or powder from gloves on the surface of the slides will make them unacceptable. Slides must be cleaned before use by dispensing a drop of lens cleaner on the slide and vigorously rubbing dry with lens paper.
2. Insert the Diff-Safe blood dispenser into the center of the tube stopper of an EDTA anti-coagulated blood sample. Gently invert the tube of blood several times so that it is well mixed. NOTE: An alternative to the Diff-Safe blood dispenser is to fill a microhematocrit tube with blood or use a wooden applicator stick to dispense on to the slide. Due to safety issues most labs use the Diff-Safe blood dispenser.
3. Invert the tube of EDTA blood 10 times.
4. Turn the tube upside down and press against the slide to place a drop of blood 2 mm in diameter on one of the slides. The drop should be in the center line approximately 1/4 inch from the frosted edge of the slide. ***Make the smear immediately after you have applied the drop of blood.***
5. Hold the slide with the drop of blood at the opposite end with the thumb and forefinger of your non-dominant hand. Grasp the spreader slide similarly with your dominant hand.
6. Rest the left end of the spreader slide at a 30° angle just in front of the drop of blood. Draw the spreader slide BACKWARDS until it just touches the drop of blood. To make a short smear, hold the spreader at a steeper angle, to make a longer smear, decrease the angle. Allow the drop of blood to spread in the angle between the slide and the spreader. Not spreading the blood evenly will cause a rounded feathered edge.
7. Keep the spreader slide at the 30° angle. Push the spreader slide **rapidly** across the stationary slide with one even stroke and pressure, avoiding any jerky movements. It should extend 2/3s of the total slide area.

Note: Any pressure exerted on the spreader slide should be directed across the slide in the direction that the film is made rather than down on the stationary slide. 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. The angle will also vary the results. An angle greater than 30° makes the smear thicker; less than 30° degrees, the smear is thinner. ***Speed, angle and drop size can be varied slightly to produce a good smear.***



Note: This picture illustrates the proper procedure for making blood smears. Notice the angle and direction that the spreader slide is pulled, first **BACK** towards the drop of blood, then quickly **FORWARD** to the end of the slide. *The spreader slide is held in the dominant hand.*

8. Allow the slide to air dry. To facilitate air drying, fan the slide back and forth by holding between thumb and forefinger and waving.
9. Check for acceptability. The smear should cover approximately 3/4 of the length of the slide. The feathered edge should be either straight or bullet shaped. The preference of a straight or bullet smear is laboratory directed. It should have a *rainbow sheen* when reflecting light. The smear should be smooth the entire length of the slide with no holes, lines or grainy appearance. The slide consists of a blood smear that is exactly *one cell thick in the feathered edge* when viewed microscopically.
10. Label the blood smear with the date, patient's name (last name first) and identification number.

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RESULTS

Name _____ Date _____

Instructions for evaluation of blood smear:

1. Prepare a blood smear according to the instructions.
2. Present blood smear to your instructor for evaluation.
3. The instructor will evaluate and write the points earned in the “Yes” column. No points are awarded for any item checked “No”.
4. Students must earn a minimum of 9 points (75%) to successfully complete this exercise.
5. If the student earns less than 9 points they must continue preparing blood smears until an acceptable slide is produced.

Criteria to determine acceptability – 1 point each unless noted otherwise.	Points	Yes	No
1. Smear is smooth, no ripples due to jerky movement.	1		
2. Holes do not appear in the blood smear.	1		
3. Extends at least 2/3s of the slide. (2 points)	2		
4. Smear is spread across (side to side) both sides of the slide to the edge.	2		
5. End of smear is straight across not rounded.	2		
6. Rainbow sheen at end of the slide.	2		
7. Properly labeled.	2		
Total Points Earned	12		

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Name _____ Date _____

Points: 24

1. What are peripheral blood smears used for in the clinical laboratory? (1 point)
2. What is involved in performing the test from question 1? (1point).
3. When preparing the blood smears what size drop is used **AND** where is the drop of blood placed on the slide? (1 point)
4. Blood viscosity will vary from patient to patient and will affect the manner in which the blood smear technique is formed. Describe the two primary conditions affecting the viscosity of blood **and** how you would alter your technique to compensate for these conditions (4 points).

	Condition	Technique to Compensate
Increased Viscosity		
Decreased Viscosity		

5. List three qualities of the appearance of the “perfect” blood smear. (3 point).
 - a.
 - b.
 - c.
6. What angle should routinely be used when holding the spreader slide when preparing a blood smear (1 point).

7. List three factors which may be altered slightly to produce a good blood smear. (3 points)
 - a.
 - b.
 - c.

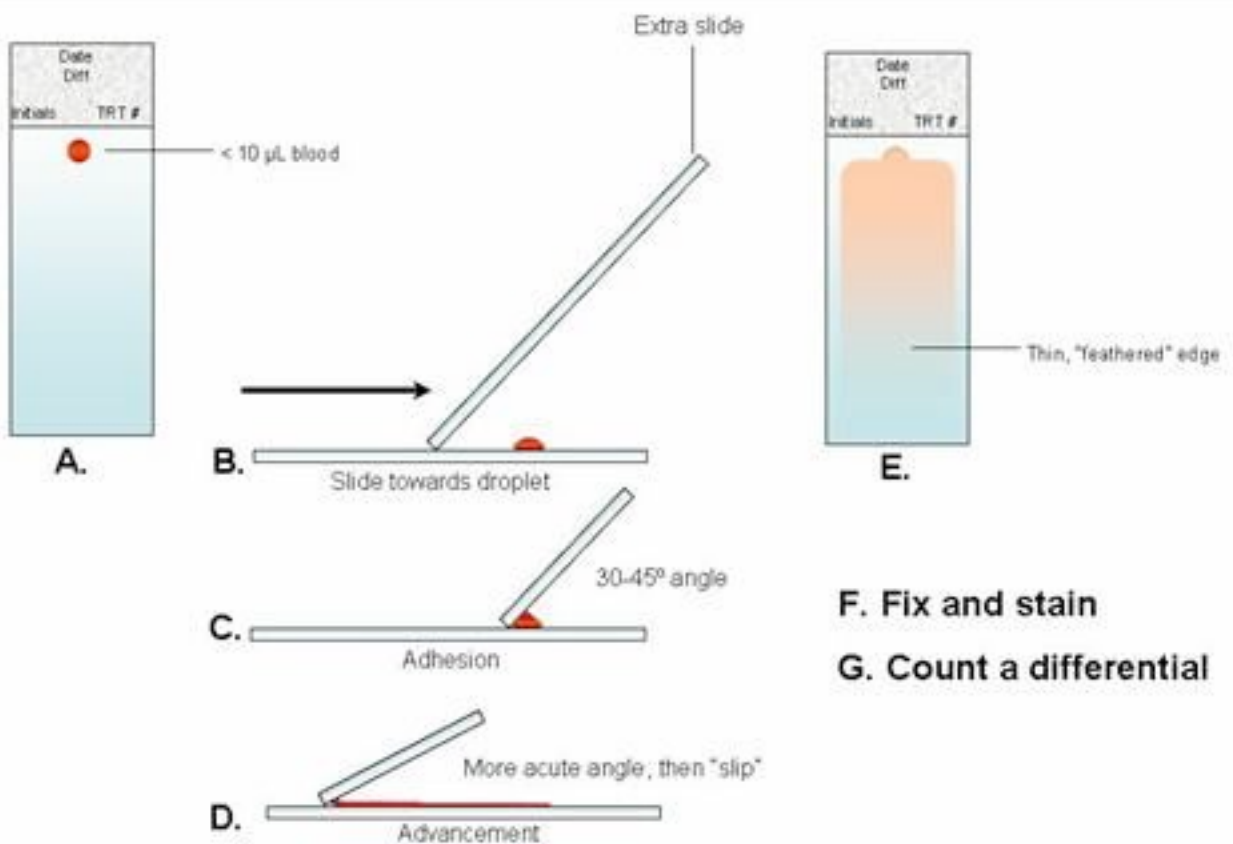
8. List three precautions to follow when preparing blood smears. (3 points)
 - a.
 - b.
 - c.

9. List five causes of poor blood smear preparation. (5 points)
 - a.
 - b.
 - c.
 - d.
 - e.

10. **Briefly** describe the proper procedure for making a blood smear. (2 points).

PREPARING BLOOD SMEARS

The following two pictures illustrate the correct way to make a blood smear. The positioning and angle of the slides is **CRITICAL**. **If your smear does not end**, i.e. the blood goes all the way to the end of the slide, **increase the angle** of your spreader slide. **If the smear is too short**, **decrease the angle** of the spreader slide. Grip the spreader slide as close to the end as possible and apply equal pressure across. Unequal pressure will result in a partial smear.



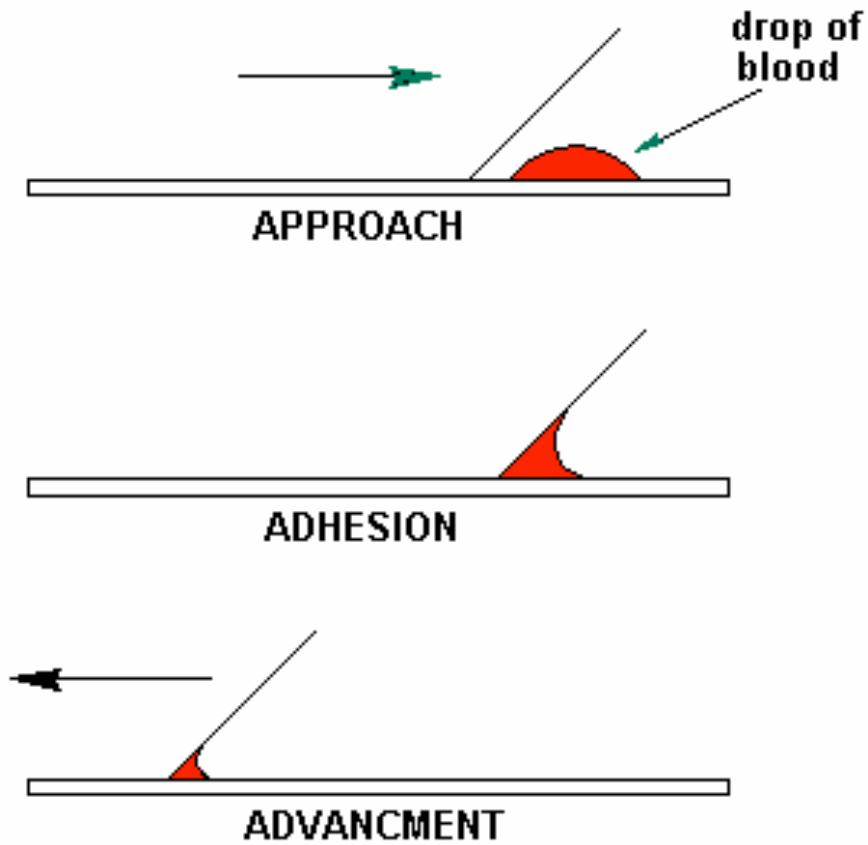


Fig. 13 - How to prepare a blood smear.