EXERCISE 9: UNOPETTES, BLOOD SMEARS AND CAPILLARY PUNCTURE

Textbook: Chapter 8 Blood Collection Equipment
Chapter 10 Procedures for Collecting Capillary Blood Specimens

Skills: 20 points

Objectives:
1. Perform a capillary puncture using aseptic technique.
2. Describe the components and contents of the Unopette blood dilution system.
3. List 4 tests which may be performed on blood collected into the Unopette.
4. Collect blood in the Unopette pipet without the presence of bubbles.
5. Accurately and completely transfers blood from pipet into Unopette reservoir without spilling diluent.
6. State the sources of errors when preparing blood dilutions using the Unopette system.
7. Prepares an acceptable blood smear for routine Hematological studies using proper blood drop size, correct angle of spreader slide, and adequate speed.
8. Describe how a “differential” is performed.
9. List three qualities of the appearance of the “perfect” blood smear.
10. Discuss procedural errors which may occur when making blood smears.

Unopette Blood Dilution System

With some microcollections the phlebotomist is required to set up the test at the patient's bedside by collecting the sample and then making dilutions of the sample. A micropipette and dilution system are used to make this task easier.

The brand name for this type of system is the Unopette test system which consists of a self-filling capillary pipette consisting of a straight, thin-wall, uniform-bore plastic capillary tube fitted into a plastic holder and a plastic reservoir containing a pre-measured volume of reagent for diluting. The reservoir is punctured to open access to the reagent.
The capillary puncture is performed and blood is allowed to fill the capillary tube without bubbles. If bubbles are present the tube must be discarded and another one used. After filling the pipette it is rinsed in the reservoir containing the diluting fluid. These devices are used for such tests as platelet counts, hemoglobin determinations, and WBC and RBC counts.

Sources of Error:

1. Squeezing the reservoir and spilling some of the liquid will give an inaccurate result.
2. While wiping the outside of the capillary pipette, any blood drawn out of the tube will cause a falsely decreased result.
3. Not wiping the blood from the outside of the capillary pipette will cause a falsely increased result.
4. Bubbles in the capillary pipette or incomplete filling of the capillary pipette will cause falsely decreased results.
5. Using a reservoir that has a punctured diaphragm will give inaccurate results. The fluid may have evaporated to the extent that results will be affected.

Preparation and Use of Blood Smears

It may also be necessary for the phlebotomist to prepare blood smears from capillary blood collected at the patient's bedside. 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 Technician or Technologist will count 100 WBCs noting how many of each kind is present. This aids the physician in diagnosing viral infections, bacterial infections and certain blood disorders such as leukemia. Blood smears are also made for such tests as malarial smears and special hematology procedures.

A good blood smear has a feathered edge that is nearly square and a rainbow sheen when reflecting the light. The perfect slide consists of a smear that 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.

Blood viscosity (thickness of blood) will vary from patient to patient. Two main conditions will affect the viscosity of the blood. Polycythemia (too many red blood cells) will increase the viscosity of the blood. Anemia (abnormally low number of red blood cells) will cause a decrease in the viscosity. When the viscosity is increased, a thinner slide is needed. To accomplish this, decrease the angle between the spreader and the slide. 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.

It takes considerable practice to make good slides consistently. There are slide-making devices on the market. These are too large to carry with you and are designed to work with automated differential reading instruments. Some of these devices make an actual wedge smear while others spin the slide to create a monolayer of cells over the entire slide. The handmade wedge or thin slide is the most commonly prepared blood film.

VISIT: http://irt.austin.cc.tx.us/webdev/MLTtemp/mltvids.htm to view the video on preparing blood smears.
**Procedure A: Unopettes**

**Materials:**

1. Capillary puncture equipment (refer to Lab Exercise 4).
2. Unopette reservoir
3. Unopette pipet
4. Unopette shield
5. Kimwipes
6. Biowipes

**Instructions:**

1. Assemble all equipment and perform a venipuncture on the patient.
2. Place the reservoir on a flat surface, holding it securely with one hand. Using the other hand, firmly push the tip of the pipette shield through the plastic diaphragm in the neck of the reservoir. Once it is punctured remove the shield and set aside.
3. Cover the stopper on the purple top and remove.
4. Tilt the tube of blood until the blood is at the edge of the tube, hold the pipette almost horizontal while touching the tip of the pipette to the patient's blood. The pipette will fill by capillary action. The filling is complete and will stop automatically when the blood reaches the end of the capillary bore in the neck of the pipette.
5. Do not quit filling until pipette is full. Take great care to avoid air bubbles. If air bubbles do occur quickly refill another capillary pipet.
6. Wipe any excess blood from the outside of the capillary pipette, making sure that you do not touch the tip and cause some of the sample to be accidentally removed from the bore of the pipette.
7. Squeeze the reservoir slightly to force out some of the air. Take care not to expel any liquid. Maintain pressure on the reservoir.
8. Cover opening of overflow chamber on the back of the pipette with index finger and carefully insert and seat the pipette back into the reservoir neck.
9. Release pressure on the reservoir. Then remove finger from pipette opening. Negative pressure will draw blood into the diluent.
10. Gently squeeze the reservoir two to three times to rinse capillary bore of blood, forcing diluent into, but not out of, overflow chamber. Pressure is released each time to return the mixture to the reservoir.
11. Place index finger over upper opening and gently invert five to six times to thoroughly mix blood with diluent.
12. Label the specimen and take to the laboratory.
Procedure B: Blood Smears

Materials:

1. Blood filled capillary tube OR purple top with diff-safe dispenser
2. Glass microscope slides.
3. Biowipes
4. 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. Gloves should be worn for the remaining steps of the procedure.

2. Insert the Diff-Safe blood dispenser into the center of the tube stopper of an EDTA anticoagulated blood sample. NOTE: An alternative to the Diff-Safe blood dispenser is to fill a microhematocrit tube with blood to dispense on to the slide. Due to safety issues most labs use the Diff-Safe blood dispenser.

3. Turn the tube upside down and press against the slide to place a drop of blood 1 to 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.

4. Hold the slide with the drop of blood at the opposite end with the thumb and forefinger of your nondominant hand. Grasp the spreader slide similarly with your dominant hand.

5. Rest the left end of the spreader slide at a 45-degree angle just in front of the drop of blood. Draw the spreader slide backward until it just touches the drop of blood. 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.

6. Keep the spreader slide at the 45-degree angle. Push the spreader slide rapidly across the stationary slide with one even stroke and pressure. Avoid any jerky movements.

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 45 degrees makes the smear thicker; less than 45 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.*

7. Allow the slide to air dry. To facilitate air drying, fan the slide back and forth by holding between thumb and forefinger.

8. 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.

9. Label the slide with patient name, number and date.
EXERCISE 9: UNOPETTES/BLOOD SMEARS/VENIPUNCTURE
RESULTS

Name _____________________________

Unopette________________________

Blood Smear _________________
EXERCISE 9: UNOPETTES/BLOOD SMEARS/VENIPUNCTURE

Name_______________________________   Date___________________

Points: 17

1. Describe the Unopette test system (1.5 points).

2. What must be done if you get air bubbles in the capillary tube while collecting blood for the Unopette test system (1 point).

3. According to the instructions in the procedure, how do you know when the Unopette capillary pipet is completely filled (1 point).

4. List three sources of error in the preparation of the Unopette and state whether this would cause a falsely increased or decreased result (3 points).
   A.

   B.

   C.

5. What are blood smears used for in the clinical laboratory and what is involved in performing this test (1.5 point).

6. Describe three qualities of a “good” blood smear (1.5 point).
7. 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 (2 points).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Technique to Compensate</th>
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<tbody>
<tr>
<td>Increased Viscosity</td>
<td></td>
</tr>
<tr>
<td>Decreased Viscosity</td>
<td></td>
</tr>
</tbody>
</table>

8. When preparing the blood smear where is the drop of blood placed on the slide (1 point).

9. What angle should routinely be used when holding the spreader slide when preparing a blood smear (1 point).

10. What three factors may be altered slightly to produce a good blood smear (1.5 points).

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