PROCEDURE: Parasitology – Malaria Information and Slide Preparation – Clinic information only

PURPOSE:

To provide instruction for preparing thick and thin blood films for identification and enumeration of common blood parasites.

PRINCIPLE:

Species identification of all blood parasites are usually made from two types of stained blood films: a thin film and a thick film. The thin smear preserves the structure of individual parasites with a minimum of distortion. The thick smear for malaria is preferred for diagnosis since it contains 16 to 30 times as much blood per microscopic field as does the thin smear, thus increasing the chance of detecting light infections.

SPECIMENS:

I. Whole blood is **preferred.**

   A. Smears must be prepared immediately after whole blood is drawn.

II. Anticoagulated blood

   A. Lavender top EDTA

   B. Pink top EDTA

III. Label the tube with the date and time of collection.

IV. Invert the EDTA tube several times so the blood will not coagulate.

V. The tube of preserved blood **should** be received in the laboratory within 1 hour of collection for best results.

   NOTE: If delay occurs, the RBC's may lyse, and true stippling may not be visible within the infected RBC's. Parasite morphology may also be altered.

   A. If it is anticipated that the EDTA tube will not arrive in the laboratory within 1 hour, prepare thin smears according to the following instructions and send the slides and the EDTA tube to the laboratory immediately.

PROCEDURE:

I. Clean all glass microscope slides (even those labeled as precleaned) with 70% alcohol swabs or dip in methanol. Polish with a lint free cloth, i.e., Kim Wipe or lens paper.

   A. **Preparation of thin blood smears** (See Figure 1)

      1. Prepare 4-5 separate slides.

      2. Place a small drop of blood, 2 mm in diameter, about 2 cm from the end of a pre-cleaned 3 X 1 glass slide near the frosted end.

      3. Place another slide at a 30-45° angle up to the drop, allowing the drop to spread along the contact line of the 2 slides.

         NOTE: This angle is important to prevent the leukocytes from bunching along the edges.

4. Quickly push the upper slide toward the unfrosted end of the lower slide.
   a. Use the correct amount of blood and proper spreading technique to obtain a smear with a good, feathered edge.

   b. The thickness of the smear varies on the rate of speed when pushing the slide.

      i. The slower the motion, the thinner the smear.

      ii. The thicker the smear, the structural detail of individual parasites can be difficult to visualize.

   c. A well-prepared film is thick at one end and thin at the other (one layer of evenly distributed RBCs with no cell overlap).

   d. The thin, feathered end should be at least 2 cm long, and the film should occupy the central area of the slide, with free margins on both ends.

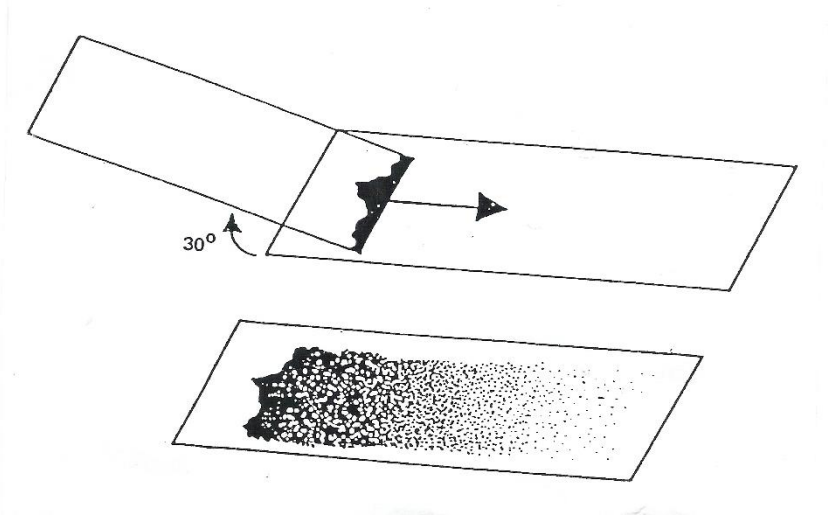

Figure 1: Preparation of thin blood smear

B. **Preparation of <u>thick</u> blood smears**

NOTE: The blood is concentrated in a small area and is several cell layers deep. During staining, the red cells are lysed, and only white blood cells, platelets, and parasite (if present) will be visible.

1. Prepare 4-5 separate slides.

2. Place two very small drops of blood on a pre-cleaned slide.

3. With an edge of a clean slide or applicator stick, and using a circular motion, mix the drops and spread the blood over an area of about 2 cm in diameter (size of a nickel) so that it has the density (when wet) that allows you to barely read newsprint placed under the smear. Do not make the smears too thick, because the blood flakes off during the staining process.

4. Continue stirring for about 30 seconds.

II. Air dry all slides in a covered box or other container to prevent dust from settling on the slides. Do <u>not</u> dry with heat.

III. Send to the laboratory immediately. **<u>DO NOT STAIN</u>**.

REFERENCES:

1. Leventhal, R., Ph.D., MBA, MT(ASCP) and Cheadle, R., MS, MT(ASCP). Medical Parasitology. A Self Instructional Text, 4th ed. F. A. Davis Company. Philadelphia. 1996.

2. Garcia, L. S. Diagnostic Medical Parasitology, 5th ed., pp 881-885. ASM Press. Washington, D.C. 2007.