Species identification of all blood parasites are usually made from two types of stained blood films: a thin film and a thick film. 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. The thin smear is used primarily for specific parasite identification. The thin smear preserves the structure of individual parasites with a minimum of distortion. These films can be made from whole or anticoagulated blood. Whole blood is preferred; however, thick and thin smears must be prepared immediately after whole blood is drawn. If anticoagulants are used, the blood must be drawn in a lavender- or pink-top (EDTA) tube. Label the tube with the date and time of collection. Invert the EDTA tube several times so the blood will not coagulate. The tube of preserved blood must be received in the laboratory within 1 hour of collection for best results. The RBCs will lyse and true stippling may not be visible within the infected RBCs. If it is anticipated that the EDTA tube will not arrive in the laboratory within 1 hour, prepare thick and thin smears according to the following instructions and send the slides and the EDTA tube to the laboratory immediately.

PREPARATION OF THICK AND THIN SMERS

Clean all glass microscope slides, even those labeled as precleaned, with 70% alcohol swabs or dip in methanol. Polish with a lint free cloth; ie, Kim Wipes or lens paper.

A. Preparation of Thin Blood Smear

1. Prepare 4 to 5 separate slides.

2. Add a small drop of blood, 2 mm in diameter, about 2 cm from the end of a precleaned 3 X 1 glass slide near the frosted end.

3. Place another slide at a 30° to 45° angle up to the drop, allowing the drop to spread along the contact line of the 2 slides. 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.
   i. Use the correct amount of blood and proper spreading technique to obtain a smear with a good feathered edge.
   ii. The thickness of the smear varies when the slide is pushed at various rates. The slower the motion, the thinner the smear. If the smear is too thick, the structural detail of individual parasites will not be observable. See figure.
   iii. A well-prepared film is thick at one end and thin at the other (1 layer of evenly distributed RBCs with no cell overlap). 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.

5. Air dry in a box or other container to prevent dust from settling on the slides. Do not dry with heat. Send to the laboratory immediately. **DO NOT STAIN.**
B. Preparation of Thick Blood Smear

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 to 5 separate slides.
2. Place 2 very small drops of blood on a precleaned 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 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.
5. Allow the smear to air dry at ambient (room) temperature in a dust-free area (slide can be placed in a petri dish with cover). Send to the laboratory immediately. **DO NOT STAIN**.

References: