

COMBINATION THICK AND THIN BLOOD FILMS (CAN BE STAINED AS EITHER)

PREANALYTICAL CONSIDERATIONS

I. Principle

The combination thick-thin blood film provides both options on one glass slide and the slide can be stained as either a thick or thin blood film. If fixed prior to staining, then the smear will be read as a thin blood film; if RBCs are lysed during staining, the preparation will be read as a thick blood film (parasites, platelets, WBCs). This combination blood film dries more rapidly than the traditional thick blood film, thus allowing staining and examination to proceed with very little waiting time for the slide(s) to dry (1,2).

II. Specimen

The specimen usually consists of fresh whole blood collected by finger puncture or of whole blood containing EDTA (0.020 g/10 ml of blood) that was collected by venipuncture and is less than 1 h old. Heparin (2 mg/10 ml of blood) or sodium citrate (0.050 g/10 ml of blood) may be used as an anticoagulant if trypanosomes or microfilariae are suspected.

III. Materials

A. Reagents

None

B. Supplies

1. Glass slides (1 by 3 in., or larger if you prefer), alcohol washed
2. Glass marker
3. Blood collection supplies (if applicable)
4. Paper with newsprint-size print
5. Applicator sticks

C. Equipment

None

ANALYTICAL CONSIDERATIONS

IV. Quality Control

- A. Visually, the smear should consist of alternating thick and thin portions throughout the length of the glass slide.
- B. One should be able to barely read newsprint through the wet or dry film.
- C. The film itself should not have any clear areas or smudges, indicating that grease or fingerprints were on the glass.
- D. Blood from a finger puncture is not recommended, since the procedure does not lend itself to "stirring" to prevent fibrin strands.

V. Procedure (Figure 1)

- A. Wear gloves when performing this procedure.
- B. Place a clean 1- by 3-in. glass microscope slide on a horizontal surface.
- C. Place a drop (30 to 40 μ l) of blood onto one end of the slide about 0.5 in. from the end.
- D. Using an applicator stick lying across the glass slide and keeping the applicator in contact with the blood and glass, rotate (do not "roll") the stick in a circular motion while moving the stick down the glass slide to the opposite end.
- E. The appearance of the blood smear should be alternate thick and thin areas of blood that cover the entire slide.
- F. Immediately place the film over some small print and be sure that the print is just barely readable.
- G. Allow the film to air dry horizontally and protected from dust for at least 30 min to 1 h. Do not attempt to speed the drying process by applying any type of heat, because the heat will fix the RBCs and they subsequently will not lyse in the staining process.
- H. **This slide can be stained as either a thick or thin blood film.**
- I. Label the slide appropriately.
- J. If staining with Giemsa (as a thick film) will be delayed for more than 3 days or if the film will be stained with Wright's stain, lyse the RBCs on the thick film by placing the slide in buffered

water (pH 7.0 to 7.2) for 10 min, remove it from the water, and place it in a vertical position to air dry.

- K. If staining with Giemsa (as a thin film), after the film is completely dry, fix it by dipping the slide into absolute methanol, and allow the film to air dry in a vertical position. If the film will be stained with Wright's stain, it does not need to be fixed. Wright's stain contains the fixative and stain in one solution.

POSTANALYTICAL CONSIDERATIONS

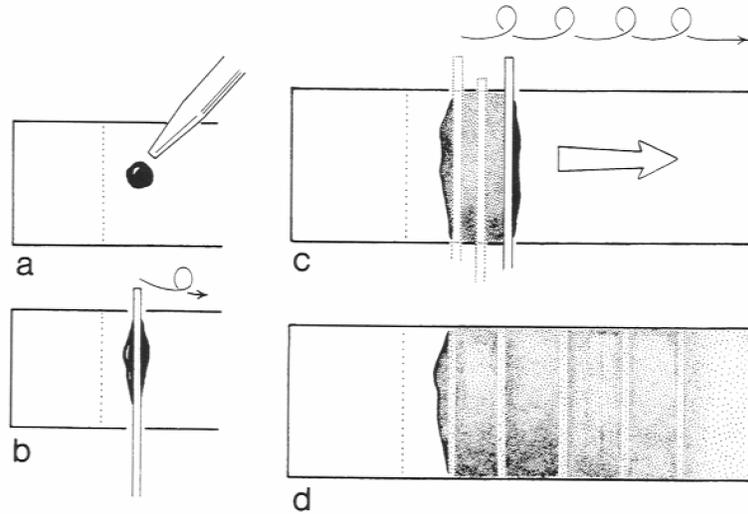
VI. Procedure Notes

- A. A diamond marking pen is recommended.
- B. An indelible ink pen can be used.
- C. Pencil can be used if the information is actually written in the thickest part of the smear (where the original drop of blood was placed).
- D. Do not use wax pencils; the material may fall off during the staining procedure.
- E. Make sure the films are protected from dust (while drying).

VII. Limitations of the Procedure

- A. A light infection may be missed in a thin film, whereas the increased volume of blood present on a thick film may allow the detection of the infection, even with a low parasitemia.
- B. If the smears are prepared from anticoagulated blood which is more than 1h old, the morphology of both parasites and infected RBCs may not be typical.
- C. **If a tube of blood containing EDTA cools to room temperature and the cap has been removed, several parasite changes can occur. The parasites within the RBCs with respond as if they were now in the mosquito after being taken in with a blood meal. The morphology of these changes in the life cycle and within the RBCs can cause confusion when examining blood films prepared from this blood.**
 - a. Stippling (Schüffner's dots) may not be visible.
 - b. The male gametocyte (if present) may exflagellate.
 - c. The ookinetes of *Plasmodium* species other than *P. falciparum* may develop as if they were in the mosquito and may mimic the crescent-shaped gametocytes of *P. falciparum*.
- D. Identification to species, particularly between *P. ovale* and *P. vivax* and between the ring forms of *P. falciparum* and *Babesia* spp., may be impossible without examining one of the slides stained as a thin blood film. Also, *Trypanosoma cruzi* trypomastigotes are frequently distorted in thick films.
- E. Excess stain deposition on the film may be confusing and make the detection of organisms difficult.

Figure 1: Method of thick-thin combination blood film preparation. (a) Position of drop of EDTA blood; (b) position of applicator stick in contact with blood and glass slide; (c) rotation of applicator stick; and (d) completed thick-thin combination blood film prior to staining. (Illustration by Sharon Belkin)(From reference 2, with permission).



SUPPLEMENTAL READING

1. **Garcia, L.S.** 2001. *Diagnostic Medical Parasitology*, ed 4., ASM Press, Washington, D.C.
2. **Garcia, L.S.** 1999. *Practical Guide to Diagnostic Parasitology*, ASM Press, Washington, D.C.

APPENDIX

Blood stain reagents available from Medical Chemical Corporation are as follows:

REAGENT	CATALOG NUMBER	SIZE AND CATALOG NUMBER	
Giemsa stain	591A	591A-16 oz	16 oz
Giemsa buffer, pH 6.8*	592A	592A-32 oz	32 oz
Methanol	107B	107B-16 oz 107B-1 gal 107B-5 gal	16 oz 1 gal 5 gal
Wright's Dip Stat #1 Fixative	301	301- 16 oz 301 – 1 gal	16 oz 1 gal
Wright's Dip Stat #2 Fixative (Eosinate Stain)	302	302-16 oz 302-1 gal	16 oz 1 gal
Wright's Dip Stat #3 Fixative (Polychrome Stain)	303	303-16 oz 303-1 gal	16 oz 1 gal
Wright's Dip Stat Stain Kit (Fixative, Eosinate, Polychrome, and Rinse Solutions)	300K	4 x 8 oz Kit	
Wright's stain (requires buffer 593A)	926A	926A – 32 oz 926A – 1 gal	32 oz 1 gal
Wright's buffer	593A	593A – 32 oz 593A – 1 gal	32 oz 1 gal
Wright's stain, one step	929A	929A – 32 oz 929A – 1 gal	32 oz 1 gal

*Check Web site for Giemsa Buffers at pH 7.0 and 7.2.