Buffy Coat Smears for the Diagnosis of *Plasmodium* spp. Infections

PREANALYTICAL CONSIDERATIONS

I. Principle

When thick and thin blood films are negative in a suspect malaria patient, the buffy coat method is recommended as an adjunct method. Blood stages can be concentrated using centrifugation of blood collected in EDTA anticoagulant, placed in a Wintrobe tube. Some studies indicate that infected red blood cells (RBCs) containing older trophozoites of *Plasmodium vivax, P. ovale*, and possibly *P. malariae* tend to concentrate above the RBC layer. After centrifugation of the Wintrobe tube, the buffy coat containing white blood cells (WBCs) and platelets and the layer of RBCs immediately below the buffy coat are used to prepare both thick and thin films. This is a more sensitive method to detect malarial parasites than thick or thin films, particularly when the level of parasitemia is low. **NOTE**: Although capillary tubes have been used in the past, this approach requires cutting the tube; possible exposure to blood and shards of glass makes use of the capillary tube unacceptable (5)

II. Specimen

The specimen consists of fresh whole blood collected using EDTA anticoagulant (0.020 g/10 ml of blood; the blood is collected by venipuncture and is less than 1 to 2 h old (1, 3, 4).

III. Materials

A. Reagents

See Appendix

B. Supplies

1. Glass slides (1 by 3 in. [1 in. = 2.54 cm], or larger if you prefer), alcohol washed
2. Glass marker
3. Blood collection supplies (if applicable)
4. Wintrobe tubes
5. Disposable 9" Pasteur pipettes (glass preferred)
6. Test tubes (size not critical)
7. Gauze
8. Applicator sticks
9. Appropriate staining reagents (Giemsa recommended)

C. Equipment

1. Centrifuge (tabletop or floor model)
2. Binocular microscope with 10x, 40x, and 100x objectives (or the approximate magnifications for low power, high dry power, and oil immersion examination).
3. Oculars should be 10x. Some worker prefers 5x; however overall smaller magnification may make final organism identifications more difficult.

ANALYTICAL CONSIDERATIONS

IV. Quality Control

A. Visually, the smears should resemble routine thick and thin blood films.

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. Concentrate known positive specimens and verify organism/blood cell recovery at least quarterly and particularly after the centrifuge has been recalibrated. A positive *P. vivax* specimen is recommended; however, if the WBCs, platelets, and RBCs concentrate properly (based on smear examination), then this is acceptable.

E. Although there is not universal agreement, the microscope should be recalibrated once each year. This recommendation should be considered with heavy use or if the microscope has been bumped or moved multiple times. If the microscope does not receive heavy use, then recalibration is not required on a yearly basis.

F. Record all QC results.

V. Procedure

A. Wear gloves when performing this procedure
B. Fill a Wintrobe tube with blood collected using EDTA anticoagulant.
C. Centrifuge at 300 x g for 15 min.
D. Remove and discard the plasma from the Wintrobe tube, leaving a small amount just above the buffy coat.
E. Aspirate the remaining plasma, the buffy coat, and the RBCs (IMMEDIATELY BELOW THE BUFFY COAT) and transfer this aliquot to a clean test tube.
F. Mix the aliquot thoroughly (slowly and carefully to prevent bubbles).
G. Prepare both thin films and thick films, allow to dry, and stain.
H. Dip the dry thick film in acetone twice, allow it to dry, and then stain; this will prevent the films from sloughing off during staining (2). The thick films can be dried in a 37ºC incubator for 10-15 min (2).

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 (works best for thin films, but not thick films)
D. Do not use wax pencils.
E. Make sure the films are protected from dust (while drying).
F. Buffy coat smears have been shown to have 100 to 200 times more organisms per microscopic field than in regular thick films (6).

VII. Limitations of the Procedure

A. It is important to select the proper area of the Wintrobe tube when taking the aliquot from which films will be prepared.
B. Both thick and thin film preparations from the buffy coat require the same techniques used for routine thick and thin blood film preparation.
C. Some organisms may be lost and some parasite distortion may occur due to high speed centrifugation; however, the sensitivity is increased over that seen with routine thick and thin blood films.
D. The number of platelets present will require very careful examination of the smears to prevent false positive results.

References:

1. **Gerber, J.E., T.E. Ukena, L. Cote, J.M. Wyllie, and W.C. Winn, Jr.** 1981. Exflagellation of malarial parasites in human peripheral blood. J. Clin. Microbiol. **13:**236-237.
2. **Hira, P.R., and K. Behbehani**. 1984. Acetone-fixed, Giemsa-stained thick films for the diagnosis of malaria. Annals of Tropical Medicine and Parasitology **78:**77-70.
3. **Kokoskin, E.** 2001. *The Malaria Manual*. McGill University Centre for Tropical Diseases, Montreal, Quebec.
4. **National Committee for Clinical Laboratory Standards**. 2000. Use of Blood Film Examination for Parasites, Approved guideline M15-A. National Committee for Clinical Laboratory Standards, Wayne, PA.
5 **Woo, P.T.K., and L. Hauck**. 1987. The haematocrit centrifuge smear technique for the detection of mammalian *Plasmodium*. Transactions of the Royal Society of Tropical Medicine and Hygiene **81**:727-728.
6. **Worth, R.M.** 1964. The heparinized capillary tube as an epidemiologic tool. II. Concentration of blood parasites by centrifugation. American Journal of Hygiene **80**:70-74.

General References for Protocols for Blood Collection and Processing

1. **Garcia, L.S.** 2001. *Diagnostic Medical Parasitology*, 4th ed., ASM Press, Washington, DC.
2. **Garcia, L.S.** 1999. Practical Guide to Diagnostic Parasitology, ASM Press, Washington, DC.
3. **Isenberg, H.D**. (ed). 1998. *Essential Procedures for Clinical Microbiology*. ASM Press, Washington, D.C.
4 **Isenberg, H.D.** (ed). 1992. *Clinical Microbiology Procedures Handbook*. ASM Press, Washington, DC.

5. **Isenberg, H.D.** (ed). 1994. Clinical Microbiology Procedures Handbook, Supplement #1. ASM Press, Washington, DC.

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 16 oz 107B-1 gal 1 gal 107B-5 gal 5 gal
Wright's Dip Stat #1 Fixative	301	301- 16 oz 16 oz 301 – 1 gal 1 gal
Wright's Dip Stat #2 Fixative (Eosinate Stain)	302	302-16 oz 16 oz 302-1 gal 1 gal
Wright's Dip Stat #3 Fixative (Polychrome Stain)	303	303-16 oz 16 oz 303-1 gal 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 32 oz 926A – 1 gal 1 gal
Wright's buffer	593A	593A – 32 oz 32 oz 593A – 1 gal 1 gal
Wright's stain, one step	929A	929A – 32 oz 32 oz 929A – 1 gal 1 gal

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