

DEPMEDS LABORATORY PROCEDURES
DEPARTMENT OF CLINICAL SUPPORT SERVICES
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL
FORT SAM HOUSTON, TEXAS 78234-6137

MCCS-HCM

STANDING OPERATING PROCEDURE

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MALARIA STAINING AND IDENTIFICATION TECHNIQUES

1. INTRODUCTION:

Examination of peripheral blood for the presence of malarial parasites includes proper specimen preparation, proper staining procedures, and proper microscopic analysis. A good patient history is also extremely helpful when available.

2. PRINCIPLE:

The best time to make blood smears for the diagnosis of malaria is halfway between paroxysms. Usually during a paroxysm (a severe attack or increase in violence of a disease, usually recurring periodically), the merozoite-filled RBCs rupture releasing free merozoites and malarial pigment into the bloodstream. Both thick and thin blood smears are required for a thorough examination. Detection is most likely on thick films, and thin films are usually necessary for identification. If parasites are not found in the initial blood specimen/smears, it is advisable to take additional thick and thin smears every 6 to 8 hours, for as long as 48 hours if necessary.

3. SPECIMEN:

- a. Capillary blood from **fingersticks** is the specimen of choice, with at least three thick and thin smears requested.
- b. If blood comes in EDTA, make 6 thick and 6 thin smears immediately.
- c. If the EDTA-treated blood yields indeterminate results upon examination, request another specimen.

NOTE: Heparin is not recommended because of frequently developing platelet clumps that interfere with the morphonological interpretation of platelets and platelets count estimates.

4. REAGENTS AND MATERIALS:

- a. Wright-Giemsa Stain (Quick-Stain).
- b. Absolute Methanol (Methyl alcohol).
- c. Distilled water.
- d. 3 urine cups or Coplin jars.
- e. 1-ml disposable pipette.
- f. Known positive controls slides from Trend Scientific, Inc. (Cat # QC11-01).

5. PROCEDURE:

- a. Preparation of slides.
 - (1) Use clean microscopic slides only.
 - (2) For thick smears, place 6 drops of blood on the slide, two each in the three points of triangular shape. Use the corner of another slide to mix the drops in a circular motion and spread them out to the approximate size of a dime. Allow the slide to air dry at least one hour, preferably overnight.

NOTE: Only air-dry the thick smears. Do not heat the thick smears with a flame or slide warmer, this tends to fix the RBCs.

- (3) For thin smears, place a drop of blood on a slide, and using another slide, streak out the blood as in making a differential smear (see enclosure). Try to get a good-feathered edge if at all possible; then allow to air dry.
- b. Staining the thin smear.

NOTE: A positive control slide should be stained with all malaria specimens when available.

- (1) Dip slide in a Coplin jar of Absolute Methanol 5 times, one second per dip.
- (2) Place slide in a Coplin jar of Wright-Giemsa Stain (Quick-Stain) for 10 seconds.

- (3) Place slide in a Coplin jar of distilled water for 20 seconds or more (for desired color balance).
- (4) Drain slide and allow to air dry in an upright position (standing on end).

c. Staining the thick smear.

- (1) Place slide on flat surface.
- (2) Carefully overlay the entire slide with distilled water.
- (3) Allow the water to lyse the RBCs (approximately 3 minutes) and remove excess water from slide. Allow slide to air dry completely before staining.

NOTE: A positive control slide should be stained with all malaria specimens when available.

- (4) Place slide in a Coplin jar of Wright-Giemsa stain (Quick-Stain) for 10 seconds.
- (5) Place slide in a Coplin jar of distilled water for 20 seconds or more (for desired color balance).
- (6) Drain slide and allow to air dry in an upright position (standing on end).

6. RESULTS:

a. Examination.

- (1) Examine the stained slides under the microscope with oil immersion (1000x).
- (2) Examine thick smears thoroughly for the presence of malarial parasites. Cytoplasm of the Plasmodia stains robin egg blue and the nuclear chromatin crimson or violet. Examine at least 100 oil immersion fields on each thick film.
- (3) The thin films are used primarily for identification of the Plasmodia species. However, even if no parasites are discovered on the thick films, the thin films must still be examined. Examine at least 200 fields on each thin smear.

- (4) When malarial parasites are observed, identify the organism using the flow chart below.
- (5) The results obtained from all positive malarial slide preparations should be phoned to the attending physician as soon as possible.

7. QUALITY CONTROL:

- a. 100 oil immersion fields of the thick smears and 200 oil immersion fields of the thin smears must be examined.
- b. When a positive sample has been found in the lab, make up as many smears as possible to use as future positive controls. Malaria parasites do not hold up well over a long period without fixation; unfixed controls may become inadequate in a few days.
- c. A known positive control slide (Trend Scientific, Inc., CAT # QC11-01) is to be stained along with the patient smears. Examine the control slide for characteristic color and morphology.

8. SAFETY:

- a. Giemsa stain is poisonous and may be fatal or cause blindness if swallowed. If swallowed, induce vomiting and repeat until clear.
- b. Reagents are flammable.

9. REFERENCES:

- a. NCCLS. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture. Villanova, PA H3-A4; 1998.
- b. Garcia, L. et al., Diagnostic Medical Parasitology 4th Ed.. New York: Elsevier Science Publishing Co., 2001.
- c. CAMCO Quick-Stain Set (Wright-Giemsa) Product Insert, Baxter Scientific Products, McGraw Park, IL.