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Section: Parasitology Manual	Subject Title: Laboratory Procedures for Blood and Tissue Parasites	
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Malaria - *Plasmodium* spp. and *Babesia* spp.

PROCEDURE

Preparation and staining of thick and thin smears.

PRINCIPLE

A number of parasites may be recovered in a blood samples, either whole blood, buffy coat preparations or various methods of concentration. These include *Plasmodium*, *Babesia*, *Trypanosoma* sp., *Leishmania donovani* and microfilaria.

The simplest method for detecting malaria and babesia continues to be the blood film.

The thick film provides the greatest sensitivity and should be performed on all malaria requests. Thin films have a lower sensitivity and are primarily used to make the species identification.

SPECIMEN

- whole blood in EDTA or ACD anticoagulant
- fingerprick whole blood sample

MATERIALS

Reagents:

absolute methanol

Giemsa stock solution (commercial product) (see appendix)

phosphate diluent buffer (see appendix)

Field's stain (commercial product)

Equipment:

Light microscope with ocular micrometer and set for Kohler illumination

Pasteur pipettes

Glass slides

Sharps container

Staining jars

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Important safety note: It is important to remember that universal precautions should be used at all time when handling blood or body fluids.

QUALITY CONTROL

1. A QC slide of *P. falciparum* or *P. vivax* should be included with a run of stain slides at least monthly and whenever a new batch of Giemsa stain is acquired.
2. The stock solution of Giemsa is stable for many years but must be protected from moisture. The aqueous working solution of Giemsa stain must be prepared fresh for every staining procedure.
3. A Giemsa stock solution must have a screw lid and be adequately protected from moisture and oxygen.
4. When the smear is properly prepared and the stain correct, the background, red cells, white cells, and protozoan parasites will be as described in results.
5. The identification of Shuffner's dots in *Plasmodium ovale* and *Plasmodium vivax* depend on the pH of stain solution. Verify that the buffer is pH 7.0 to 7.2.
6. Record all QC results and report any "out-of-control" results to lab director for action.
7. The microscope should be serviced annually.
8. The biological safety cabinet (BSC) should be serviced annually or after any movement of the BSC and service dates should be recorded on the BSC.

PROCEDURE

Special Safety Note: *Blood tubes should only be opened in a running biological safety cabinet.* Any spills should be cleaned up immediately. Gloves must be worn when preparing samples and any contact with contaminated sharps or contact of blood or body fluids with broken skin should be reported immediately to the lab director and Employee Health. Malaria, Babesia, and blood-borne viruses can be transmitted from blood samples, therefore follow up of exposure is important.

Prepare thick and thin blood films using pre-cleaned grease free frosted slides.

GIEMSA-thin films

- a. A thin film is prepared exactly as one used for differential count. There should be a thin feathered end (at least 2 cm long) containing evenly distributed red blood cells with no overlap and occupying a central area of slide with margins free on both sides.
- b. Allow film to air dry. **DO NOT APPLY HEAT.**
- c. Fix the blood film in absolute methanol for one minute.
- d. Place into Giemsa stain solution for 50 minutes (1:50 dilution--see appendix)
- e. Wash by gently dipping into buffer pH 7.0 -7.2 two to three times. Note: excessive washing will decolorize the film.

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- f. Drain thoroughly in vertical position and allow to air dry.

GIEMSA thick-film

- a. Apply two or three small drops of fresh whole blood onto an alcohol clean slide
- b. With the corner of another slide using a circular motion spread the drops to cover an area approximately 2cm in diameter (Note: you should just be able to read newsprint through a thick smear).
- c. Allow the film to completely air dry (room temperature). **DO NOT APPLY HEAT.**
- d. **DO NOT FIX THICK FILM.** Place film into GIEMSA stain solution for 50 minutes (1:50 dilution-see appendix).
- e. Wash gently in buffer for one to two minutes.
- f. Air dry in vertical position.

EXAMINATION OF BLOOD SMEARS

- a. Thick and thin blood films should initially be reviewed at low power (100 x magnification) particularly at the edges of the thick and thin film where microfilaria, malaria parasites and trypanosomes may be concentrated.
- b. Thick film should then be examined systematically beginning in the center of the film and moving in a defined fashion out from the center. At least 200 oil immersion fields should be reviewed (magnification x 1000).
- c. Thin films should be examined systematically back and forth across the feathered end of the film for at least 300 oil immersion fields (magnification x 1000).

Expected Stain Results

A. Thin film

- a. The background should be clean and free of debris; the color of the erythrocytes is a pale grayish pink.
- b. Neutrophil leukocytes have a deep purple nuclei with well defined granules.
- c. The chromatin of malaria parasites is a deep purplish red and cytoplasm is a clear purplish blue
- d. Stippling should show up Schuffner's dots in erythrocytes containing *Plasmodium vivax* or *P. ovale* and Mauer's spots in erythrocytes containing the larger ring forms of *Plasmodium falciparum*.

B. Thick film

- a. The background should be clean and free of debris with pale mottled gray color derived from the lysed erythrocytes.
- b. Leukocyte nuclei are a deep purple

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- c. Malaria parasites are well defined with deep red chromatin and a pale purplish blue cytoplasm.
- d. In *P. vivax* and *P. ovale* infections the presence of Shuffner stippling in the ghost of the host erythrocyte can be seen.

REPORTING

The presence of malaria parasites, the species identified and the level of parasitemia should be reported immediately to the attending physician and the laboratory director. High levels of parasitemia (>1% or >50,000 parasites/ul)) are critical and should be reported immediately to the attending physician and the laboratory director.

Method of Determining Parasitemia in thick blood films:

- a. Count parasites and leukocytes separately
- b. If after 200 leukocytes have been counted, 10 or more parasites have been identified, record the results in the record form indicating the number of parasites seen per 200 leukocytes.
- c. If after 200 leukocytes have been counted nine or less parasites have been counted, continue counting until 500 leukocytes have been counted and record the parasites observed per 500 leukocytes counted.
- d. Report the parasite count in parasites per microlitre in relationship to the leukocyte count by the following formula; the parasites per microlitre is equal to:

$$\frac{\# \text{ of parasites X white blood cell count per ul}}{\# \text{ of leukocytes counted}}$$

If a white blood cell count is not available assume a white cell count of 6000/ul.

LIMITATIONS

1. It may take several thick and thin blood smears to exclude the diagnosis of malaria, particularly in semi-immune individuals or on individuals on chemosuppressive therapy.
2. The sensitivity of the thick smear is estimated to be 10-100 parasites/ul and therefore low parasitemias may be missed.
3. It may be difficult to determine the species identification in cases with low numbers of circulating ring forms and in cases of mixed infections.

If blood samples are old or if patients have received partial therapy the morphology of the parasites may be altered making species identification difficult.

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