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Section: <b>Parasitology Manual</b>	Subject Title: <b>Laboratory Procedures for Blood and Tissue Parasites</b>	
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### Field's Stain

#### Alternative Stain for Thick Smears

- a) Place thick smear in Solution A for 5 seconds.
- b) Wash by gently rinsing in tap water for 10 seconds.
- c) Gently shake the slide to remove excess water.
- c) Dip in Solution B for 5 second.
- d) Wash as in b) for 10 seconds.
- e) Stand on end and air dry.

#### EXAMINATION OF BLOOD SMEARS

- 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.
- 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).
- 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

- The background should be clean and free of debris; the color of the erythrocytes is a pale grayish pink.
- 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*.

##### C. Thick film

- The background should be clean and free of debris with pale mottled gray color derived from the lysed erythrocytes.
- Leukocyte nuclei are a deep purple
- Malaria parasites are well defined with deep red chromatin and a pale purplish blue cytoplasm.
- In *P. vivax* and *P. ovale* infections the presence of Shuffner stippling in the ghost of the host erythrocyte can be seen.

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## 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:

- Count parasites and leukocytes separately
- 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.
- 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.
- 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:  

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

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

## LIMITATIONS

- 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.
- The sensitivity of the thick smear is estimated to be 10-100 parasites/ul and therefore low parasitemias may be missed.
- 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.

## REFERENCES

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