## TITLE: Malarial Smears

### PRINCIPLE

Malaria is caused by a genus of parasites called *Plasmodium*. There are four species of the genus that normally infect humans. These species are *Plasmodium vivax, P. ovale, P. malariae,* and *P. falciparum.* Once inside the cell, the parasites grow at the expense of the host cells. The parasite goes through several growth stages within the red cell until it divides into erythrocytic merozoites. The red cell ruptures and the merozoites are released. These merozoites can then infect other cells. The rupturing of the red cells is associated with the chill and fever characteristics of clinical malaria. Thus in order to find the maximum number of parasites inside the red cells it is best to make the smears 10 – 12 hours after paroxysms. The chill-fever cycle associated with *P. malariae* is 72 hours. The other three species exhibit a 48 hour chill-fever cycle. Man can be infected by more than one species or by different broods of the same species leading to irregular paroxysms. The malarial parasites contain stains produced from hemoglobin. The cytoplasm of the parasites can be confused with a platelet lying on top of a red cell if one does not look carefully. The malarial parasite is one cause of extracorpuscular hemolytic anemia. The parasite can also cause leukopenia and neutropenia by depressing the marrow-especially its granulocytic producing or marrow releasing capacity. Included is the identification of *Babesia* species also in the same manner.

 It is important to report the level of parasitemia as well when the smears are examined and found to be positive for malaria.

 Requests for malaria indicates that the presence of any blood parasite should be considered and examination for babesiosis, trypanosomiasis and filariasis is necessary.

### CLINICAL SIGNIFICANCE

Because of, the potential for drug resistance in some of the *Plasmodium* species, particularly *P. falciparum,* it is important that every positive smear be reported in the same manner. This allows the parasitemia to be followed after therapy has been initiated. Where the patient is hospitalized, monitoring should be performed at 24, 48, and 72 hours after initiating the therapy. Generally the parasitemia will drop quickly within the first few hours; however in cases of drug resistance, the level may actually increase over time.

# PERSONNEL

Medical Technologists, Technicians, Pathologist, Infection Prevention and Phlebotomists

# SPECIMEN

Patient Preparation: No special patient preparation by laboratory personnel.

Type of Specimen: Preferably thick and thin (wedge) smears made at the bedside from the finger. Alternately, EDTA anticoagulated whole blood may be used to make the smears. Fresh blood less than 1 hour old is best.

Handling Precautions: Handle all specimens using Standard Precautions.

# REAGENTS

Wright Giemsa stain, Buffer (phosphate – 6.8 pH).

Immersion oil

Distilled water, (pH of 6.8 – 7.2)

# EQUIPMENT

Glass slides, absolutely clean and grease free

Slide stainer

Microscope

Ocular Micrometer: available in the pathologist’s office, for use in determining size of blood borne parasites. (Dr. Abraham has one available to use on his scope). All calibration and care for the micrometer is in the pathologists’ care.

# CALIBRATION

Micrometer calibration is the responsibility of the pathologist.

# QUALITY CONTROL

 **Stain quality is assessed and documented on a daily basis. See 4840-HE-0572 Aerospray Hematology Pro Use and Maintenance.**

**STEPWISE PROCEDURE**

1. Thick and thin finger stick or freshly drawn EDTA smears are obtained. The smears are made the same as differential smears. Make several of each type, thick and thin. Thick smears are made by taking a drop of blood and spreading it over a small portion of the glass slide: Approximately no larger than the size of a dime.

 Example:

|  |  |
| --- | --- |
| Pt.Name and date |   |

1. The smears are permitted to dry thoroughly. This is especially important for the thick smears.
2. After thoroughly drying, the thick smear must be laked or dehemoglobinized (lysis of the red cells). This can be accomplished as follows:
3. Gently place several drops of distilled water on the dried smear. pH of water should be 6.8 to 7.2. Ours is about 7.0 (test with pH test strip to check).
4. Permit the water to stay on the slide for a minimum of 2 minutes. It generally takes longer.
5. Gently tilt the slide and allow the hemoglobin containing water to run off the slide.
6. Let the smears dry thoroughly; then put them on the slide stainer.
7. Stain the smears the same way as a differential smear.
8. Read the smears using 100 x oil immersion.
9. **For thick or thin smears, examine at least 300 oil immersion fields, minimum per slide.**
10. **For positive smears**: Count the number of parasitized RBC’s per 100 RBC’s in 10 different oil immersion fields. If the number of parasites is high, count the number of infected RBC’s per 200 RBC’s (instead of 1000). Report the percentage counted. (0.5%, 1.0%, etc.) # of parasitized RBC’s x 100 = % parasitemia

 1000 (or 200)

1. All malarial smears are reviewed by a minimum of 2 Technologists, with the assistance from a pathologist and/or Infection Prevention Manager for positive smears.

**CALCULATIONS**

Report the percentage of infected RBC’s per 100 RBC’s counted. Example: 5 infected cells counted in 1000 RBC’s results in a parasitemia of 0.5%. 5/1000 x 100 = 0.5%

**REPORTING RESULTS**

Report results through the LIS with the use of keypads for result entry.

 **Malaria Smear:** None Seen

 Present

An interpretation comes up if the smears are negative, that suggests further collections as recommended by our governing agencies. This interpretive data is built as a canned message that fires upon the result entered into Soft.

**Parasite Id: C**lick in the result field and choose the **“comment”** @PLSM. The text appears: Plasmodium species at a \_% parasitemia. Fill in your results in the \_ field. **Add a canned message that smears were reviewed by a pathologist (@PATH) and add another Canned Message that “patient sample has been sent to IDPH for the final identification” (@IDPH).**

 **Example: *Plasmodium species*** present at a 0.5% parasitemia.

**NOTE: Results of initially positive malaria smears need to be reported to the physician or clinical personnel immediately, and documented in LIS as we do in with our critical values by using the Call tab in the Result Worklist..**

***Positive results for malaria require notification to the Infection Control Manager, and minimum 1 mL Lavender EDTA whole blood sent to IDPH-Springfield.\*\* Results must be reported to the IDPH within 7 days. Upon certification of results, print a report for Infection Prevention and call them to let them know to pick up the report in the Lab. Do not send the report via in-house mailers, we have a limited time frame.***

**Requirements for malarial parasite identification by IDPH include 1 mL Lavender EDTA whole blood; complete the IDPH Communicable Disease Test Request form. All patient demographics must be completed: patient’s name, date of birth, ethnicity, date of collection, date of onset, name of test required and travel history (country and dates). Specimen must be refrigerated, please refer to 4840-Sendouts-165, for specific mailing instructions.**

**Upon receipt of the results from IDPH, we have a binder to put chart copy and IDPH report in, and a copy of IDPH results goes into Micro on the Infection Prevention clipboard.**

**PROCEDURAL NOTES**

*P. falciparum* development takes place extravascularly in internal organs. Thus a first negative report may not reflect a real negative. A first negative report only states that no parasites were in the peripheral blood at that time.

After the first set of negative smears, samples should be taken at intervals of 6 – 8 hours for at least 3 successive days. Another recommendation is to draw a new specimen every 24 hours for a total of three days.

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