BLOOD PARASITE SCREEN – THICK AND THIN SMEARS

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PURPOSE

To provide instruction for the preparation and evaluation of peripheral blood smears for blood-borne parasites, and to estimate the percentage of malarial or babesial parasitemia.

BACKGROUND

Thick and thin blood smears are microscopically scanned for blood parasites responsible for Malaria, Babesiosis, Trypanosomiasis, and Filariasis. Positive slides with malaria or babesia parasites are reported with the percentage of parasitemia. The parasitemia level is used to monitor effective therapy and possible drug resistance.

All positive and negative slides are reviewed by a pathologist. If positive, the slide and sample are then submitted to the Washington State Public Health Lab for full identification and speciation. **Note:** Malaria is a reportable disease for the County Health Department and must be reported within 3 days of the initial positive result to the patient's county of residence.

Thick and thin smears should be prepared within one hour of collection for optimal identification of blood parasites.

RELATED DOCUMENTS

Critical Lab Value Management Policy	R-PO-AD0551
Peripheral Smear Preparation	R-W-HEM1418

SPECIMEN REQUIREMENTS

- Thick and Thin smears (2-4) from finger stick or EDTA blood specimen within 1 hour of collection. Smears prepared over 1 hour, or delayed in delivery will not be rejected but will be reported with a comment.
- The optimal time for sample collection is during or soon after a fever spike; however specimen collection should not be delayed while awaiting fever spikes.
- A single set of slides may not reveal organisms. Successive smears every 8-12 hours for up to three days are sometimes necessary. Blood samples should be taken before any anti-malarial or anti-parasitic drugs are used to ensure demonstration of the organisms.

EQUIPMENT/SUPPLIES

- Harleco Phosphate Buffer, pH 7.0
- Sigma-Aldrich Giemsa Stain
- Methanol
- Microscope, timer, and tally counter. (**Note:** An ocular micrometer is available in Microbiology, if needed.
- Microbiologics Blood Parasite Control Slide, (known positive for Malaria, Babesia, or Trypanosomes) or a previously prepared positive patient slide to be used as a positive control.

- Blood Parasite worksheet for result and comment documentation.

QUALITY CONTROL

1. A commercial or previously prepared positive control slide and a negative control slide prepared from a patient with normal medical history are stained with the patient slides. Review slides for presence of parasites on the positive control, and for quality of staining on the negative control. See Step 1 below, Scanning the Smears.
    - Note: If a positive control slide is unavailable for testing, review previous known positive slides, or review the reference materials available in the department.

2. Two techs must review both thick and thin smears before reporting.

3. All positive and negative slides are submitted for pathologist's review.

INSTRUCTIONS

Note: Malaria is one of the few parasitic infections that can be immediately life-threatening. Preparation, staining, and review of the slides are performed Stat with minimal delay. Positive results are called as Critical Values.

Thin smear preparation

(**Note:** All slides require two patient identifiers.)

1. **All Sites:**
    - Thin Smears: Prepare 3-4 patient smears as you would a peripheral blood smear optimally within one hour of specimen collection.
    - Air-dry for 10 minutes.
    - If not at SJMC, Place in slide holder for shipping and send to SJMC stat. If at SJMC, proceed to step 2.

2. **SJMC only:**
    - Prepare a negative control slide prepared from a patient with normal medical history and air dry.
    - Make 3-4 thin patient smears if not previously prepared and air dry.
    - Fix the patient THIN smears and the negative QC slide in methanol for 5-7 minutes and air dry. Use fresh methanol for each slide batch.
    - Select a positive Blood Parasite Control Slide for staining. This slide is already fixed in methanol by the manufacturer or when previously prepared.
    - Continue with preparation of the thick smears.

Thick smear preparation

1. **All Sites:**
    - Thick Smears only: Place a drop of blood on a slide and spread to the size of a dime using the tip of the transfer pipette or capillary tube. Newsprint should be legible through the smear. Make 3-4 slides. **Note:** Overly thick smears may dislodge from the glass during shipping.
    - Air-dry until slide is dry enough for transport. **NOTE: Thick smears are never fixed with methanol. The RBCs are intended to lyse when stained for the thick smears only.**
    - Notify SJMC Hematology if slides were prepared after 1 hour from time of collection. An LIS comment will be added to the results to notify the patient's physician. Smears collected over 1 hour, or delayed in delivery will not be rejected.

- If not at SJMC, place in slide holder for shipping and send to SJMC stat along with an appropriately labeled aliquot of the original specimen.

2. **SJMC only:**
   - Ensure that slides have been air dried for at least two hours from preparation in order to prevent separation of the smear from the slide during staining.

Stain Preparation and Buffer pH documentation

1. Buffer pH: Confirm the pH of the Harleco pH 7.0 Phosphate Buffer and document results on the blood parasite worksheet.
2. Stain Preparation: Prepare working Giemsa stain using Sigma-Aldrich Giemsa. Do not use Harleco Giemsa as this has a methanol base and will fix the **thick** slides in error.
3. Add 2 ml stock Sigma-Aldrich Giemsa to 38 ml Harleco pH 7.0 Phosphate Buffer in a Coplin jar. Stability: Make fresh, each 8 hour shift, when needed.

Staining the Slides

1. Stain the 2 QC slides, 2 thick and 2 thin patient slides in the Working Giemsa for 30 minutes, Reserve additional slides for restaining, if necessary.
2. Remove the slides from the jar and allow the stain to run off.
3. Check the thick smears: The RBC's should lyse. Note: If not lysed, slides may have been previously fixed with methanol at preparation, or the working Giemsa may have been prepared with a methanol based stain. New slides must be prepared.
4. Fill a coplin jar with the phosphate buffer and gently wash each slide individually by dipping in the buffer, allowing to drain back into the jar. Avoid heavy rinsing as the specimen may detach from the slide.
5. Air-dry slides at room temperature.

Scanning the Smears

1. CONTROL SMEARS: Scan the positive and negative control slides.
   - Using the negative control, check for excessive debris that may be confused with malarial / babesial ring-forms. Check for correct stain coloring of the RBCs which should appear pale pink.
   - Using the positive slide, review the color of the cytoplasm for parasites found, which should be pale blue. The chromatin dot of ring-forms should be dark-blue/black. Locate and confirm the parasite on the slide.

2. PATIENT SMEARS: (Thick and Thin)
   - Scan the thin slides using the 10X and 40X dry objectives looking for large worm-like microfilaria parasites. Rule out fiber contaminants. Digital images are available for comparison. Scan the entire slide.
   - Thick and Thin slides: Scan a minimum of 300 oil-immersion fields using the 100X lens to detect Malaria, Babesia, and Trypanosomes.

3. PARISITEMIA: (If indicated using Thin smears)
   - If Malaria or Babesia parasites are present, count the number of infected red cells seen within 1000 red cells. Calculate the percentage of parasitemia using the following formula:

$$\frac{\text{\# of malaria parasites seen}}{\text{1000 red cells}} \times 100 = \text{\% parasitemia}$$

4. Call and document positive results as a Critical value. **Note:** Two techs must review slides prior to reporting.

5. For all orders, submit a thick and thin slide for Pathology Review. The path review order will reflex when blood parasite results are verified.

6. Send all positive slides to the State Health Department for identification.

Sending Positive Slides for Parasite Identification

1. Select a positive thick and thin smear to send to the Washington State Health Department, Parasitology Department, for parasite identification. An aliquot of the EDTA specimen should also be sent, if possible, but is not required.

2. Have Specimen Center order the send-out test for Blood Parasite Identification in the LIS system.

3. Complete the State Health Lab requisition, located in the Blood Parasite binder. Enter the patient information and patient travel history. This information is required, and the provider may need to be contacted. Make a copy of the original requisition and place in the Blood Parasite binder.

4. Place the slides, sample, and requisition in a send-out bag for the lab courier. Notify the specimen center coordinator to send samples ASAP with the earliest day-shift courier. Evening/Night Shift: If the provider insists on stat speciation, please contact the on call clinical pathologist to determine whether the specimen should be sent to the University of Washington.

5. Notify the MT-Coordinator or microbiology manager for all positive Malaria results. Malaria must be reported to the County Health department within 3 days of reporting positive results.

6. Refer inquiries regarding drug resistance or treatment to a pathologist, microbiology manager, or MT-Coordinator, so that an infectious disease specialist may be contacted.

LIMITATIONS

- A negative finding on one set of smears does not rule out malaria infection.
- Slides made >1 hour from collection can result in a false negative result. Testing should still be performed if specimen is older than 1 hour.
- Follow up parasitemia evaluations are recommended at 24, 48 and 72 hours after Initial treatment.

REPORTING RESULTS

1. Document all results/calculations, comments on the Blood Parasite worksheet.
2. Report test components: Seen/Not Seen
3. MAL%: Report the percent parasitemia from positive malaria/babesia slides.
4. For positive parasite screens, follow Critical Lab Value Management Policy and document the call in LIS.
5. For slides made >1 hour after collection, add the comment: “Due to the age of the specimen received, negative results are not conclusive and should be repeated on smears prepared from blood no older than one hour.”

INTERPRETATION OF PARASITEMIA RESULTS

Note: Interpretation of malarial parasitemia results are appended to the patient report when the results are verified.

Parasitemia	Clinical Correlation
0.0001-0.0004%	Number of organisms required for a positive Thick film (sensitivity).
0.002%	Patients may be symptomatic below this level.
0.2%	Level above which immune patients will exhibit symptoms.
2%	Maximum parasitemia of P. vivax and P.ovale. (infect young RBC's only)
2-5%	Hyperparasitemia, severe malaria, increased mortality.
10% or greater	Exchange transfusion may be considered, high mortality

Resistance Definitions	Comments
SENSITIVE	At start of therapy, parasites cleared by day 6. No evidence of reappearance of parasites for up to 28 days
RESISTANCE TYPE I	After therapy, parasites have cleared for two consecutive days (the latest being 6 days: reappearance of parasites follows. Patient should be monitored for a period of days, especially if drug-resistant P. falciparum is suspected.
RESISTANCE TYPE II	Within 48 hours of treatment, marked reduction of parasitemia to <25% of pre-treatment count. No subsequent clearing of parasitemia by day 6.
RESISTANCE TYPE III	Modest reduction in parasitemia may be seen; no change or increase in parasitemia seen during 1st 48 hours after treatment; no clearing of parasites. Blood films may show overall parasite increase.

REFERENCES

1. Garcia, L. S. 2001. Diagnostic Medical Parasitology, 4th ed., ASM Press, Washington, D.C.
2. Navy Environmental Health Center, Laboratory Diagnostic Techniques, Malaria Prevention and Control, Appendix 3. www.vnh.org/Malaria.
3. Diagnostic Procedures for Blood Specimens. www.dpd.cdc.gov.
4. Determination of Parasitemia Protocol, Garcia, L.S. www.dpd.cdc.gov.