Malaria and Blood Parasites **Technical Procedure #1240.t**

Principle

Blood parasites, including Plasmodium sp. Babesia sp., Ehrlichia sp., Borrelia sp., Trypanosomes, and microfilaria can be seen on Wright stained peripheral blood smears. Both thick and thin smears are used. The thick smear preparation serves to concentrate the parasites extracellularly in greater numbers, while the thin smear preparations are scanned for the intracellular presentation of the parasite to aid in speciation.

Specimen

Peripheral blood smears (thick and thin) are prepared from an EDTA anticoagulated sample. Smears should be made within 2 hours of collection. Finger stick smears are acceptable.

Reagents

A Wrights stain (thin smears). Automated stainers such as the BCI slide stainer are acceptable for staining thin smears

B Giemsa Stain Solution (thick smears) Sigma-Aldrich Accustain Giemsa Stain, GS500-500ml

C Giemsa Working Solution

1 Prepare a 1:20 dilution of Giemsa Stain Solution with prepared Volu-Sol Buffer (pH 7.0-7.2). 2.5 ml Giemsa Stain Solution and 47.5 ml Volu-Sol Buffer mixed in a 50 ml Coplin jar.

2 Giemsa working solution is stable for up to 2 weeks. Prepare fresh working solution if current reagent is not dated or is older than 2 weeks.

D Buffered distilled water

1 Fill a coplin jar ¾ full with prepared Volu-Sol Hemastain Buffer (pH 6.7 - 7.2). A new coplin jar of buffered distilled water must be prepared each time thick smears are stained.

2 Test with pH strips or with a pH meter and, if necessary, adjust the pH to the correct range using HCl or NaOH, available in the Chemistry Dept.

Note:	It is important to keep the pH of the buffer solution and wash water at approximately 7.0 in order to demonstrate sharp structural details, such as Schuffner's dots.

Equipment

A Glass slides

B Transfer pipettes

C Coplin jars

D pH strips or meter

E Pipettes and graduated cylinders

F Ocular micrometer if necessary

Quality Control

A Each time a blood parasite thick smear stain is performed, a known positive slide is included in order to verify blood parasite staining characteristics.

Positive slides: At the time of a positive blood parasite smear, extra thin smears are made, labelled as "+ blood parasite", dated, allowed to dry thoroughly, fixed with methanol and stored in a closed slide box.

Procedure

A Prepare Thin smears

1 Make six wedge smears. Let dry for five minutes.

2 Stain Thin Smears with Sedona Wright Stain on BC slide stainer.

B Prepare Thick Smears

1 Using a transfer pipette, place a drop of well-mixed blood on a clean slide. Spread the drop with the side of the pipette tip until the blood is thin enough to see print through. Repeat for a total of four slides. (If smear is made too thick, it will wash off during staining.)

2 Allow the thick smears to completely dry at room temperature until they no longer shine. This may take 8-12 hours.

3 Stain Thick Smears

a. Prepare a coplin jar of Giemsa Working Solution and a fresh jar of buffered distilled water.

b. Place two of the dry thick smears and one of positive control slides in the Giemsa Working Solution for 60 minutes.

c. After 60 minutes in the stain, gently rinse the smears in the coplin jar of buffered distilled water by placing slides in coplin jar for 3-5 minutes.

d. Dry at room temperature.

C Microscopic evaluation

NOTE:	It is recommended that two CLS's should be involved in the microscopic evaluation.

1 An Ocular micrometer is available for measuring parasites.

2 Examine the entire thin and thick smear on 10x for the presence of microfilaria.

3 Examine thin and thick smears and the quality control slide under 100x oil immersion.

4 Quality Control Slides

a. The quality control slides should show blood parasites with expected staining characteristics.

5 Thin Smears

a. The thin smears should show correct staining for both leukocytes and erythrocytes. If staining is not acceptable, prepare new smears, fresh stain and repeat staining procedure

b. Thin smears must be scanned under 10x objective in its entirety for presence of microfilaria.

Microfilaria are worm-like organisms in peripheral blood smears that range in size from 150 to 300 um. They can be sheathed or unsheathed, and will have body nuclei in varying patterns.

c. Each smear must be examined for 10 min. (total 300 fields or more) under 100x oil immersion objective for presence of blood parasites or intracellular malaria. If no blood parasites are observed, continue to the thick smears.

d. **Trypanasoma** sp. are extracellular flagellated parasites present in peripheral blood. They range in size from 8-30 um. A kinetoplast is present at one end, and nucleus in the middle, and an undulating membrane along the length of the parasite.

e. **Ehrlichia** sp.parasites are seen as membrane bound compartments in WBC's called morulae. They are rare and variably sized basophilic inclusions. A buffy coat slide may increase yield.

f. **Borrelia** sp are seen as extracellular spirochetes in the peripheral blood smear. They are long, very fine, spiral shaped parasites.

g. **Babesia** sp. can often be confused with Plasmodium sp. RBC's may contain many ring forms. The classic Maltese Cross is a tetrad formation of the parasite.

h. **Plasmodium** organisms have characteristic morphological features. To differentiate from artifacts, look for a dark pink chromatin, blue cytoplasm and brown malarial pigment (may not be evident in ring forms).

i. A platelet superimposed on a RBC appears surrounded by a halo. Compare with other platelets.

i. When blood films are positive for malaria parasites, the level of parasitemia must be reported along with the preliminary organism identification.

i. Count the number of organisms present in 1000 RBCS, counting 500 RBCs on each of 2 thin smears.

ii. Calculate the percentage of infected RBCs.

$$\% \text{ Infected} = \frac{\text{\# of infected RBCs X 100}}{\text{1000 cells counted}}$$

iii. Report the result as a percent.

NOTE: Examine the thin smears and turn out a preliminary report for negative smears "Preliminary results: No blood parasite organisms noted on peripheral thin smear examination." Use canned text "HPRE"

6 Thick Smears are for Plasmodium, Babesia, or any RBC inclusion parasite examination.

a. The thick smears should show staining of leukocytes and platelets. The leukocytes, although slightly distorted, should show characteristic staining. The erythrocytes should lyse completely during the staining process.

b. Examine the thick smears (each smear should be examined for 10 minutes to total 300 fields or more).

c. Blood parasites, if present will be distorted and condensed. Plasmodium sp. will have blue cytoplasm with bright pink chromatin dots.

Reporting Results

A A Misc worksheet is pulled and the results of both patient and control are recorded on worksheet.

B Negative smears:

1 Report N (Negative for blood parasites), then go to free text comments field. Enter "No blood parasite organisms noted on peripheral smear examination." Use canned text "HNEG".

2 File the stained slides in the malaria slide box.

C Positive Smears for malaria (Thin or Thick Smears)

1 Report P (Positive), then go to free text comments field. Enter "Malarial organisms seen on peripheral smear examination, species to be determined. [ ] % RBCs infected. "(Use canned text:"POS")

2 Call patient's physician and document call in the LIS using MPHONE canned text.

3 Save positive slides for Pathology Review and Public Health confirmation.

D Positive Smears for blood parasites (Thin or Thick Smears)

1 Report P (Positive), then go to free text comments field. Enter "Blood parasites seen on peripheral smear examination".

2 Call patient's physician and document call in the LIS using MPHONE canned text.

3 Save the positive slide for Pathology Review and Public Health confirmation.

Follow-up for All First Time Positive Smears

A If the patient is an inpatient or clinic patient at UCDMC:

1 Report the positive result verbally, including all relevant information (patient name, medical record #, clinic or ward.).

a. On Monday-Friday, day shift, notify the Infection Control Department (#3377, then select Nurse Epidemiologist).

b. On off hours or on weekends or holidays, notify the Infection Control Nursing Supervisor (pager # 916-816-5364).

c. Fax results to infection control (4-0100) OR print from LIS to "Infect" printer.

d. Document the notification in the LIS under patient requisition, stating the person notified, and the date and time of verbal and faxed notification.

B If the patient is a PCN or outreach client:

1 The result must be faxed to the Health Department of the patient's county of residence (available from the patient's physician).

2 Document this step in the LIS under patient requisition, indicating the date, time, person notified.

C Send to Public Health for confirmation

1 Fill out the printed Public Health submission slip with patient information, healthcare provider information and specimen information.

2 Place at least one stained thick and thin smear and one unstained thick and thin smear into a plastic slide box.

3 Wrap the slip around the box with "PUBLIC HEALTH DEPT" heading showing.

4 Send to Microbiology dept. for delivery to Public Health.

Note:	Specimens are picked up between 8-8:30 am by Public Health.

D Final Report (Public Health Department Report)

1 Make two copies of report.

2 Label copies appropriately in red ink in the right lower corner one as “chart copy” and the other as “lab copy”.

3 Send Chart Copy to SARC for scanning to medical records. Medical records will scan results into EMR.

4 Attach Lab Copy to Malaria worksheet and file with Fluid worksheets.

5 Report parasite species in the LIS under the originating request in the following manner:

a. If from a CBC request, enter under Comments.

i. "Final Report from Department of Public Health: _____." Use canned text “FINAL”

ii. “Interim Report from from Department of Public Health: _____.” Use canned text “INTERIM”

Reference Range

No malarial organisms or blood parasites noted on peripheral smear examination

References

A Ash, L, Orihel, T, Atlas of Human Parasitology, 3rd Ed, ASCP Press, 1990.

B Linnette, E, Spaulding, E and Truant J; Manual of Clinical Microbiology;2nd Ed.; p 605-611; American Society for Microbiology; Washington DC; 1974.

C Manual of Microscopic Diagnosis of Malaria; Scientific Publication #46Pan American Health Organisation; Washington DC; 1960.

D NCCLS; Laboratory Diagnosis of Blood-borne Parasitic Diseases: Approved Guideline; NCCLS Document M15-A; Vol 20, Approved June 2000.

E Reporting Diseases and Conditions; Administrative Procedure 115.A; UCDMC Department of Pathology Procedures.

F Center for Disease Control www.cdc.gov/parasites

G Modern Pathology 2004 17, 512-517 *Characteristic peripheral blood findings in human ehrlichiosis.*

H Centers for Disease Control and Prevention (CDC) *http://www.cdc.gov/parasites*

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Procedure History

Date	Written/Revised By	Revision	Approved Date	Approved By
8/85		new	8/1/85	N Levy MD
9/92	J Jeffries	qc, format	9/8/92	E Larkin MD
6/94	J Jeffries	minor, format	7/94	E Larkin MD
11/96	J Jeffries	rev. for CAP requirements: 300 fields/doc of phys. call	11/25/96	E Larkin MD
4/97	J Jeffries	rev for reporting requirements	4/14/97	E Larkin MD
			12/30/97	C Miller MD
			10/20/98	E Larkin MD
			12/17/99	E :Larkin MD
			10/3/00	E Larkin MD
			10/17/01	E Larkin MD
11/02	J Cannon	MS Word	11/05/02	E Larkin MD
			10/17/03	E Larkin MD
10/17/04	J Cannon	Reporting format	10/24/04	E Larkin MD
			10/23/05	K Janatpour MD
9/15/06	J Cannon	Change Giemsa stain source	11/06/06	D Dwyre MD
		Annual Review	11/05/07	D Dwyre MD
		Annual Review	07/03/08	D Dwyre MD
		Annual Review	10/27/09	D Dwyre MD
07/2010	L Gandy	Minor Change	07/2010	D Dwyre MD
11/10	L Freeman	Added micrometer	11/19/10	D Dwyre MD
		Biannual Review	8/24/12	D Dwyre, MD

3/5/14	L Freeman	Updated pH Minor change		
10/14	L Gandy	Changed to include blood parasites	10/22/2014	D Dwyre, MD