Principle

Blood parasites, including Plasmodium sp. Babesia sp., Trypanosomes, and microfilaria can be seen on Giemsa 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. Ehrlichia sp., Borrelia sp. can be seen on Wright stained peripheral blood smears. Only thin smears need to be evaluated for Ehrlichia sp. and Borrelia sp. blood parasites.

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

# Giemsa Stain Solution Sigma-Aldrich Accustain Giemsa Stain, GS500-500ml

# Phosphate buffer solution pH 7.00 Fluka Analytical 73173-1L. Expires one year from open date. Sore at room temperature.

# Triton X-100 Sigma-Aldrich X100-5ml.

## Prepare 5% Triton X-100 solution.

### Pre warm 95.0 ml DIH2O to 570C. Slowly add entire 5 ml bottle of Triton X-100 (solution is very thick). Swirl to mix.

### This solution is stable indefinitely at room temperature.

# Giemsa Working Solution. Stable for 24 hours at room temperature.

## Prepare solution

### 49 ml Phosphate buffer solution pH 7.0. Check the pH before preparing working solution and record on worksheet.

### 1 ml Giemsa Stain solution

### 2 drops 5% Triton X-100

# Methanol EMD MX0485-3 4L. Stable indefinitely.

# Sedona Lab Products Hemastain 21-105

# VoluSol buffer hemastain buffer VHB-4.4 gm. 1 envelope dissolved into 4 L DIH2O

Note: It is important that the pH of the buffer solution is between 7.0-7.2 in order to demonstrate sharp structural details, such as Schuffner's dots.

Equipment

# Glass slides

# Transfer pipettes

# Coplin jars

# pH strips

# Pipettes and graduated cylinders

F Ocular micrometer if necessary

Quality Control

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

## To prepare positive controls use a positive patient sample.

### Make as many thing smears as possible. Allow them to dry.

### Fix in methanol for 1 minute. Allow slides to dry.

### Pack tightly in slide box. Label the box “*Giemsa + Control*” and date.

### Store at <700 C indefinitely.

### When needed, remove one slide from box and allow it to come to room temperature before staining.

Procedure

# Prepare Thin smears

## Make six wedge smears. Allow them to dry.

## Fix in methanol for 1 minute. Allow slides to dry.

# Prepare Thick Smears

## 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.)

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

# Stain Smears with Working Giemsa stain.

## Prepare a coplin jar of Giemsa Working Solution and a fresh jar of buffer solution.

## Stain the thin smears first, as the thick smears dry.

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

### After 60 minutes in the stain, gently rinse the smears in the coplin jar of buffer solution by placing slides in coplin jar for 3-5 minutes.

### Dry at room temperature.

## For identifying Borrelia sp. or Ehrlichia sp. stain thin smears with Wrights stain. Use the Hematology Lab peripheral blood stainer.

## Stain the thick smears when dry using the same working Giemsa stain and the above procedure.

# Microscopic evaluation

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

## An Ocular micrometer is available for measuring parasites.

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

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

## Quality Control Slides

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

## Thin Smears

### 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.

### 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.

### **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.

### **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. Stain with Wrights stain.

### **Borrelia** sp are seen as extracellular spirochetes in the peripheral blood smear. They are long, very fine, spiral shaped parasites. Stain with Wrights stain.

### **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.

### **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).

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

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

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

#### Calculate the percentage of infected RBCs.

% Infected = # of infected RBCs X 100

1000 cells counted

#### Report the result as a percent.

NOTE: Examine the thin smears and turn out a preliminary report for negative smears “No blood parasite organisms noted on peripheral thin smear examination.” Use canned text “BPPRELIM”

## Thick Smears are for Plasmodium, Babesia, trypanosomes, microfilaria or any other RBC inclusion parasite examination.

### 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.

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

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

Reporting Results

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

# Negative smears:

## Report N (Negative for blood parasites) in the BP Prelim result, "No blood parasite organisms noted on peripheral smear examination" will populate the field.

## File the stained slides in the malaria slide box.

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

## Report P (Positive) in the BP Prelim result,“The peripheral smear was positive for the noted blood parasite. The species will follow” will populate the field.

## Report P next to the appropriate blood parasite.

## For positive Plasmodium species report the level of parasitemia.

## Call patient's physician and document call in the LIS.

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

Follow-up for All First Time Positive Smears

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

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

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

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

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

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

# If the patient is a PCN or outreach client:

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

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

# Send to Public Health for confirmation

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

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

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

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

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

# Final Report (Public Health Department Report)

## Make two copies of report.

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

## 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.

## Report parasite species in the LIS under the originating request in the BP Species result “"Final Report from Department of Public Health:\_\_\_”

Reference Range

No malarial organisms or blood parasites noted on peripheral smear examination

References

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

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

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

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

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

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

# 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*

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 |
| 1/15 | L Gandy | Thin smears stained with Giemsa |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |