Quest Diagnostics

TRAINING UPDATE

Lab Location:	WAH	**Date Distributed:**	1/8/2013
Department:	Micro	**Due Date:**	1/22/2013

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Malaria WAH.M06 v3

Description of change(s):

Section	Reason
9 10.2.1 10.2.2	Change report to “Parasitemia followed by the % infectivity”. Changed steps on how to report the % infectivity (English Text code first then free text the rate %)

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	**Malaria**
Prepared by	Ron Master Date: 5/11/2009
Owner	Ron Master Date: 5/11/2009

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Annual Review		
Print Name	**Signature**	**Date**

Form revised 10/31/02

Quest Diagnostics Nichols Institute
Site: Washington Adventist Hospital
Title: Malaria

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Malaria, thick and thin smears	Manual	MAL

Synonyms/Abbreviations
Malaria smear, Malaria ID, Malaria Parasites, *Plasmodium* species

Department
Microbiology

Form revised 10/31/02

2. ANALYTICAL PRINCIPLE

Examination of stained peripheral blood smears is used for screening and identifying malarial parasites, *Babesia*, trypanosomes, and microfilaria. Malarial and *Babesia* parasites infect circulating red cells and undergo various stages of development within the red cell. The Wright Giemsa stain highlights morphologic features of these stages.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	None
Specimen Collection and/or Timing	Slides are to be prepared when the patient presents with symptoms of malaria, and every 6 hours for 36 hours. Specimens obtained during the febrile state yield the greatest number of parasites in circulating blood. Prepare fresh finger stick thin smears and thick smears. **Thin smears:** Collect a small drop of blood near one end of a slide, and then spread the blood over the surface with a second slide. The thin, feathered end should be at least 2 cm long, and the film should occupy the central area of the slide, with free margins on each side. **Thick smears:** Prepare by touching the slide to the drop of blood (which should be rounded up on the finger). Rotate the slide to form a circular film about the size of a dime that is made up barely visible thorough wet smear. Allow for complete air-drying of smears. Label the frosted end of the slides using a pencil. Include the patient name and accession number. The phlebotomist must hand the slides directly to a technologist. Refer to Phlebotomy procedure Malaria Smear.

Component	Special Notations
Special Collection Procedures	**Collection procedure for the Germantown Emergency Department ONLY:** Because of limitations at Germantown for fingerstick collection, Malaria specimens may be collected in an EDTA lavender tube. Smears must be made within 2 hours of collection in order to reduce distortion of the parasites and RBCs. The thin and thick smears will be prepared at the Germantown ED and all of the smears and the EDTA tube will be sent to Shady Grove via STAT courier. The thin smears will be stained and examined at Shady Grove and the preliminary report will be issued. All of the smears (both thin and thick) ALONG WITH THE EDTA TUBE will be sent to Washington Adventist for final reporting.
Other	A Malaria History Form is to be completed for each patient.

3.2 Specimen Type & Handling

Criteria		
Type -Preferred **-Other Acceptable**	Two thin and two thick smears **Note**: ONLY Germantown Emergency Department may submit an EDTA tube less than two hours old.	
Collection Container	See section 3.1	
Volume - Optimum **- Minimum**	N/A N/A	
Transport Container and Temperature	Slide holder at room temperature	
Stability & Storage Requirements	Room Temperature:	1 month slides 2 hours EDTA tube
	Refrigerated:	Unacceptable
	Frozen:	Unacceptable
Timing Considerations	N/A	
Unacceptable Specimens & Actions to Take	If specimen is too old test must not be performed. Improperly prepared or improperly labeled slides. Reject specimen and request recollection.	
Compromising Physical Characteristics	N/A	
Other Considerations	Treatment with anti-malarial or other antiparasitic drugs may reduce the sensitivity of the test.	

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents	Supplier and Catalog Number
Giemsa Stain	Harleco – 620G-75
Buffer	Alphatec Giemsa (Malaria) Stain Buffer – 033-25

4.2 Reagent Preparations and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.
Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Reagent	Giemsa Stain
Container	1 L bottle
Storage	15-30ºC
Stability	Stable until expiration date
Preparation	None

Reagent	Alphatec Giemsa (Malaria) Stain Buffer
Container	125 mL bottle
Storage	15-30ºC
Stability	Stable until expiration date
Preparation	None

5. CALIBRATORS/STANDARDS

N/A

6. QUALITY CONTROL

6.1 Controls

Appearance of blood cells is noted every time a patient's smear for malaria is performed.

Romanowsky Color Range

Chromatin of white blood cells	purple
Nuclei of parasitic protozoa	red
Basophilic cytoplasm of lymphocytes, monocytes, and parasitic protozoa	blue
Eosinophilic granules	pink
Neutrophilic granules	purple
Red blood cells	salmon pink (to bluish)
Bacteria	deep blue

Record QC results on Malaria Stain QC Form.
If controls are unacceptable do not report patient results, notify supervisor.

6.2 Control Preparations and Storage

N/A

6.3 Frequency

Each batch of patient smears is evaluated for proper staining characteristics.

6.4 Tolerance Limits

A run is rejected if the WBCs, RBCs, and platelets on the intensity control are not stained adequately.

Rejected runs must be effectively addressed by corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed.

6.5 Review Patient Data

Review patient results for unusual patterns, trends or distributions in patient results such as an unusually high percentage of abnormal results.

6.6 Documentation

Steps taken in response to QC failures must be documented.

6.7 Quality Assurance Program

N/A

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

None

7.2 Equipment

Microscope
Wescor Hematology Slide Stainer

7.3 Supplies

Immersion oil
Glass slides
Geimsa Stain
Buffer

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used.

8.1	Thin Smears
1.	Allow smear to dry thoroughly before staining.
2.	Place stain intensity control smear and patient thin smears on Wescor Hematology Slide Stainer. (see Hematology procedure for stainer instructions)
3.	Examine thin smears under 10X and 100X (oil immersion) to screen for the presence of malarial parasites, *Babesia*, microfilaria, and trypanosomes.
4.	At least 300 fields must be viewed with a 100X oil immersion lens for adequate assessment.

8.2	Thick Smears (performed at WAH)
1.	Allow smear to dry thoroughly before staining (at least 2 hours).
2.	Do not fix with alcohol or heat or dry in an incubator. Heat will prevent RBC lysis.
3.	Obtain working Giemsa solution. Into a Coplin jar add 49ml of the phosphate buffer, 1 ml of the Giemsa Blood Stain. Mix well before use.
4.	Place the thick smears directly into the working solution for 45-60 minutes. The water-based Giemsa stain disrupts the red cell membrane (laking) during the staining procedure exposing the parasites.
5.	Wash the smears by rinsing them with buffer (pH 7.0 to 7.2) for 3-5 minutes.

8.2	Thick Smears (performed at WAH)
6.	Record pH of buffer on QC sheet.
7.	Air-dry in a vertical position.

8.3	Reading
1.	Scan the smear under low power first to detect presence of microfilaria or trypanosomes.
2.	Next read under oil immersion (100X objective).
3.	At least 300 fields under oil immersion must be examined.
4.	All shifts will screen thin smears for malaria, *Babesia*, microfilaria, and trypanosomes.
5.	The WAH microbiology staff will review thin smears and perform testing on thick smears.

9. CALCULATIONS

Parasitemia: In areas of the slide where the RBCs are evenly spread out over the entire field and not overlapping count the number of infected cells per field of 200 cells. Do this on 10 different areas on each thin smear and take the average and divide by 2.

Report: "Parasitemia(INF2) _____"

Use the code INF2 and enter the number and % sign.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Smears

Smears are examined utilizing oil immersion lens (100x).

Read a minimum of 300 fields under oil immersion before determining that the thin smears are negative.

Thick smears are to be read before finalizing the report as negative. Thick smears are also a guide to the intensity of the infection. Thick smears allow a large amount of blood to be examined, increasing the detection of parasites in light infections. If parasites are detected on the thick smears, species determination must be made using the thin smear examination. This is determined by the recovery and identification of life cycle stages observed on the thin smear.

10.2 Reporting

10.2.1 General Information

Call both positive preliminary and final results to the nursing unit or physician. The call back information must be documented in the LIS.

Preliminary Reports:

If thin smears are negative, report: "Thin smear presumptive negative, thick smear and final report to follow". (NMLP1)

If thin smears are positive, report: "Presumptive positive, confirmation and identification to follow." (PMAL1)

If microfilaria or trypanosomes are seen, report their presence and send to Chantilly for confirmation.

*** Do not finalize the thin smear preliminary report in the LIS.**

Final Reports:

If negative, report: "No parasites seen. One set of blood films can not exclude the diagnosis of malaria." (NMAL1)

If positive report: 1. report genus and species for malaria

2. report the level of parasitemia

Report the percentage of cells infected on all positive Plasmodium species.

In areas of the slide where the RBCs are evenly spread out over the entire field and not overlapping count the number of infected cells per field=200 cells. Do this on 10 different areas on each thin smear and take the average and divide by 2.

Report: "Parasitemia(INF2) _____"

Use the code INF2 and enter the number and % sign.

The call back information must be documented in the LIS.
* **More than one technologist must review all initial positive malaria smears.**

Document both tech codes on the LIS workcard.

10.2.2 LIS resulting

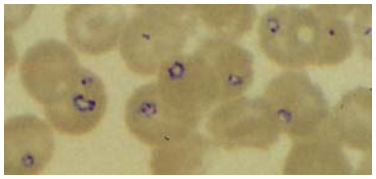
Function: Microbiology Result entry
Observation 1: Name of organism <cr>
Observation 2: <cr> M (expands to Parasitemia <cr>
;;(free text number of infectivity rate **AND include %**)
Observation 3: ;CBACK <cr> (expands to 'Called to and read back by:) ;;
add free text call documentation <cr> <cr>
Finalize report by using "/" key

* Each call must be documented. Do not delete previous call back information.

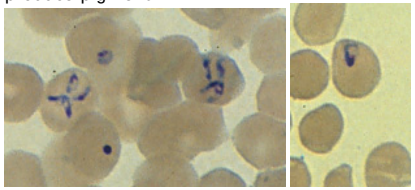
10.3 Interpretation of data

Morphological Characteristics of *Plasmodium falciparum* and *Babesia* species:

Appearance of parasite	*Plasmodium falciparum*	*Babesia* species
Size	Small (1/4 to 1/3 RBC diameter, 3-5 µm)	Tiny to small (1/8 to 1/4 RBC diameter, 1-5 µm)
Shape	Consistent oval to round ring	Pleomorphic: pear-shaped to round ring
Appliqué Forms	Common, either marginal or bulging forms	Common, either marginal or bulging forms
Number of Chromatin dots	1 to 2	1 to many ("string of pearls")
Multiple rings/RBC	Common	Common; two adjacent parasites may appear to be split into mirror images
Tetrads	No	Rarely seen
Appearance of RBCs	Normal size and shape	Normal size and shape
Parasite stages present	Ring: trophozoite with pigment (in heavy infections); banana-shaped gametocytes (rarely found)	Ring: late ring or trophozoite with no pigment, may contain a white central vacuole not seen in *Plasmodium*



A: *Babesia microti* infection, Giemsa-stained thin smear. The organisms resemble *Plasmodium falciparum*; however *Babesia* parasites present several distinguishing features: they vary more in shape and in size; and they do not produce pigment.



B.and C: Infection with *Babesia*. Giemsa-stained thin smears. Note in **B** the tetrad (left side of the image), a dividing form pathognomonic for *Babesia*. Note also the variation in size and shape of the ring stage parasites (compare **B** and **C**), and the absence of pigment.

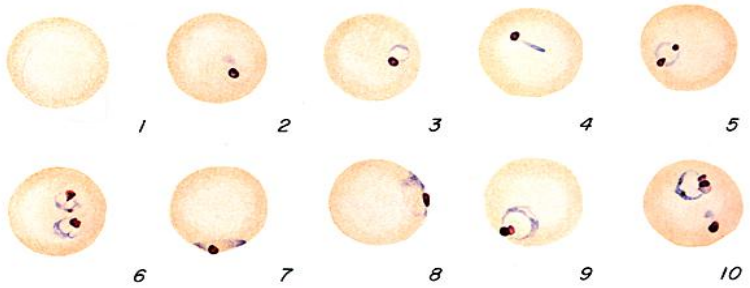
Morphology of *Plasmodium* species in Wright-Giemsa stained smears:

Characteristics	*P. falciparum*	*P. vivax*	*P. ovale*	*P. malariae*
Size and shape of infected erythrocytes	Normal size and shape	Enlarged up to twofold, may be oval	Normal to enlarged, frequently oval, may be fimbriated	Small to normal size, normal shape
Stippling	Occasional Mauer's dots, less numerous than Schuffner's	Schuffner's dots (stippling) usually present, except in rings	James' stippling, darker than Schuffner's, present in all stages, including rings	Zeiman's dots rarely seen; requires deliberate over staining
Stages seen in peripheral blood	Rings and gametocytes	All	All	All
Multiply infected erythrocytes	Common	Occasional	Occasional	Rare
Early trophozoites	Delicate ring, frequently with two small chromatin dots, often at the edge of the erythrocyte	Ring up to 1/3 diameter of the erythrocyte; larger chromatin dot than *P. falciparum*	Similar to *P. vivax*	Smaller that *P. vivax*; otherwise similar
Mature trophozoites	Not seen in peripheral blood	Amoeboid shape, fine golden brown pigment	Similar to *P. vivax* except less amoeboid, pigment darker brown	Compact cytoplasm, oval, round, or band-shaped, dark brown pigment
Schizonts	Not seen in peripheral blood	12-24 merozoites	8-12 merozoites	6-12 merozoites often radically arranged around central pigment in a rosette form
Gametocytes	Crescent of banana-shaped	Round to slightly oval	Round to slightly oval	Round to slightly oval
Most characteristic findings	Absence of mature trophozoites and schizonts; normal size of infected erythrocytes; multiply infected RBCs; appliqué forms; banana-shaped gametocytes	Enlarged infected erythrocytes; Schuffner's dots frequently present; amoeboid trophozoite; 12-14 merozoites in each schizont	Normal to enlarged, oval or fimbriated infected RBCs, James' stippling may be seen in rings; schizonts with 8-12 merozoites	Normal size of infected erythrocytes; no stippling; "band" trophozoite; rosette schizont with 6-12 merozoites

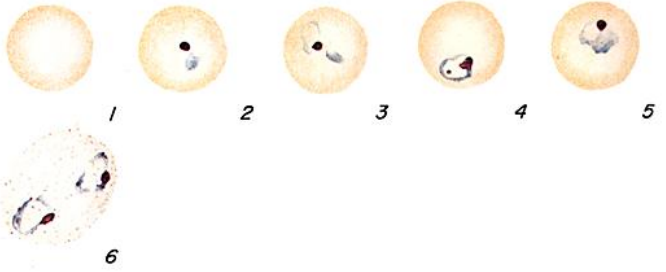
Form revised 10/31/02

Ring Stage Parasites

Plasmodium falciparum: Rings



Plasmodium vivax: Rings



Plasmodium ovale: Rings

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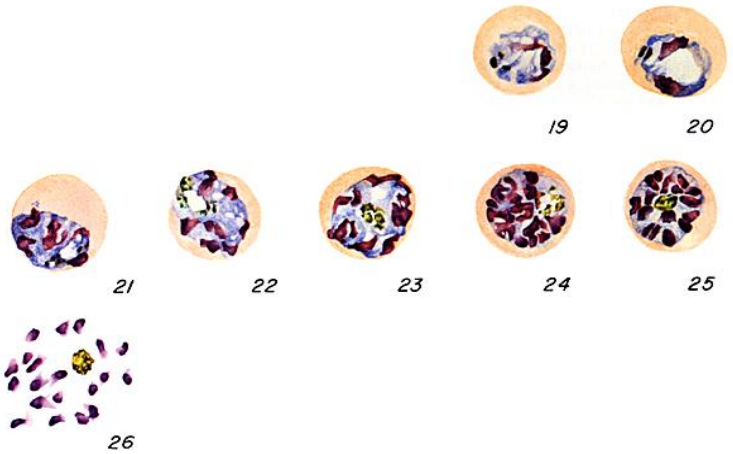
***Plasmodium malariae*: Rings**

Schizonts

***Plasmodium falciparum*: Schizonts (usually not seen in blood)**



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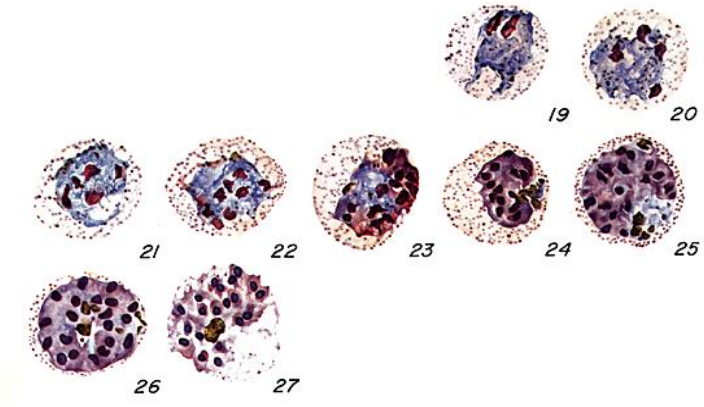
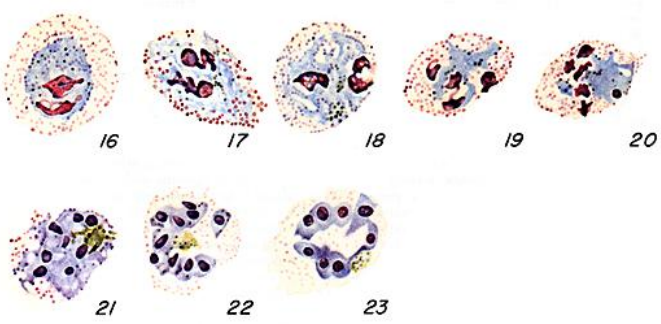
***Plasmodium vivax*: Schizonts**



***Plasmodium ovale*: Schizonts**



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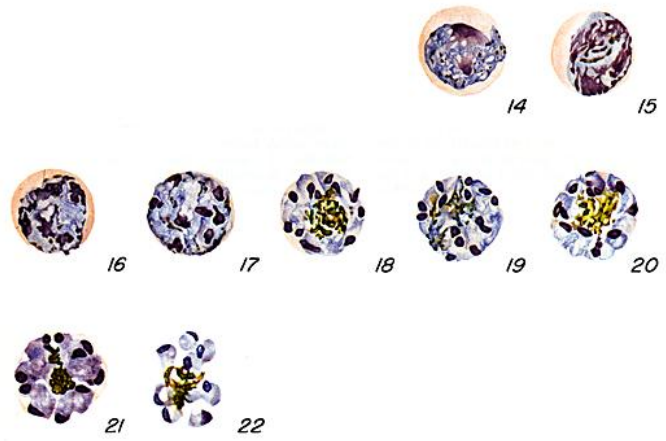
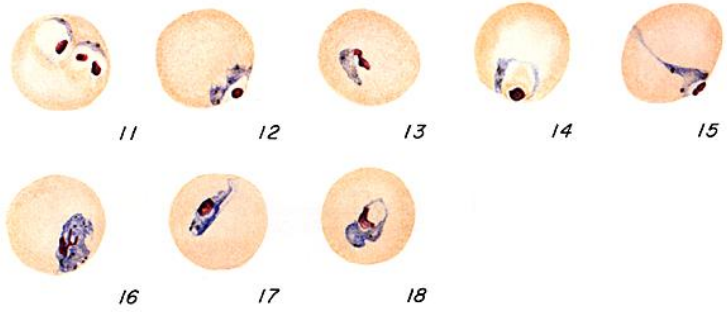
***Plasmodium malariae*: Schizonts**

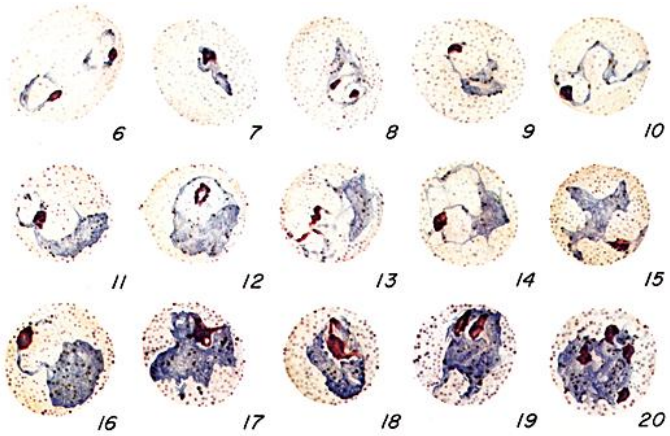


Trophozoites

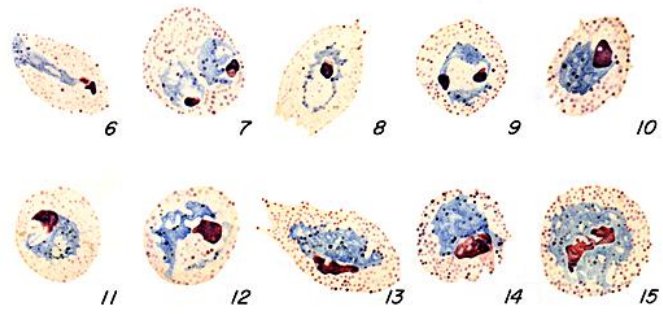
***Plasmodium falciparum*: Trophozoites (early forms may be seen but later forms usually not seen in blood)**



***Plasmodium vivax*: Trophozoites**

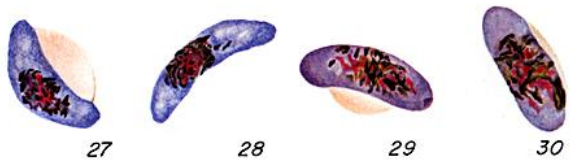


***Plasmodium ovale*: Trophozoites**

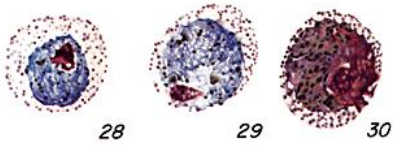


Gametocytes

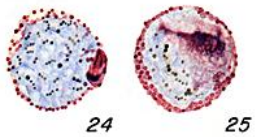
Plasmodium falciparum: Gametocytes



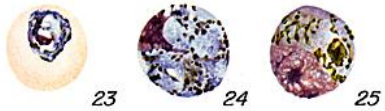
Plasmodium vivax: Gametocytes



Plasmodium ovale: Gametocytes

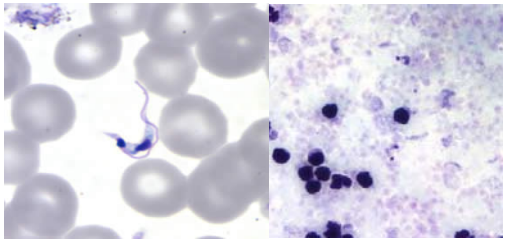


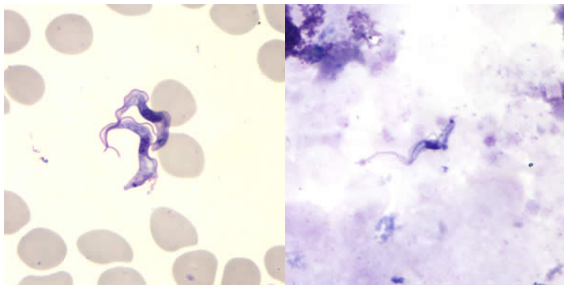
Plasmodium malariae: Gametocytes



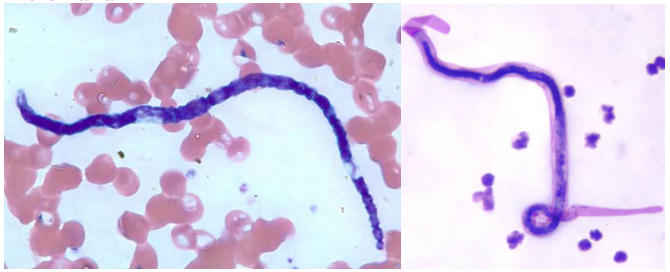
Morphology of trypanosomes and microfilaria.

Trypanosomes





Microfilaria



10.5 Rounding/Units of Measure/ Clinically Reportable Range (CRR)

N/A

10.6 Repeat Criteria and Resulting

N/A

11. EXPECTED VALUES

11.1 Reference Ranges

No parasites seen.

11.2 Critical Values

Any positive smear

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

Malaria is a disease of worldwide importance characterized by fever, anemia and splenomegaly. Although four species of the genus *Plasmodium (P. falciparum, P. vivax, P. malariae*, and *P. ovale)* ingfect humans, malaria is clinically two diseases; the benign type due to *P. vivax, P. malariae,* and *P. ovale,* and the malignant type due to *P. falciparium*.

Determination of parasitemia becomes important when therapy is initiated. The patient's parasitemia is monitored so that possible cases involving drug-resistant strains of *P. falciparium* may be detected. In those cases where the patient is hospitalized, monitoring of the parasitemia should be performed at 24, 48 and 72 hours after initiating therapy. Generally, if the malarial strain is susceptible to the therapeutic regime, the parasitemia will drop significantly within the first 24 hours (often by 50% or more).

Babesia is a malaria-like disease characterized by fever, chills, headache, lethargy and myalgia. Hemolytic anemia and hemoglobinuria are typical and may be sever. The disease is transmitted by the bite of hard ticks of the family Ixodidae. This disease is suspected when individuals have traveled through tick-infested areas and present with a malaria-like illness. The disease becomes apparent 1-3 weeks after the bite of an infectious tick. In splenectomized and immunocompromised patients this disease may be fatal. Determination of % parasitemia helps direct therapy. In severe parasitemia (>10%), exchange transfusion may be considered.

Parasitemia and Clinical Correlation

Parasitemia	Parasites /uL	Clinical Correlation
0.0001-0.0004%	5 – 20	Number of organism that are required for a positive thick film (sensitivity)
0.002%	100	Patients may be symptomatic below this level
0.2%	10,000	Level above which immune patients will exhibit systems
2%	100,000	Maximum parasitemia of *P. vivax* and *P. ovale* (infect young RBSs only)

Parasitemia	Parasites /uL	Clinical Correlation
2-5%	100,000 – 250,000	Hyperparasitemia, severe malaria, increased mortality
10%	500,000	Exchange transfusion may be considered, high mortality

13. PROCEDURE NOTES

- **FDA Status:** LDT without message
- **Validated Test Modifications:** None

Any alcohol left on the skin prior to collection may fix the red cells and then they will not clear in the staining procedure.

Do not dry smears using heat, as this will fix the red cells.

Slides prepared from EDTA blood are not optimal as they may cause distortion in the parasites, making identification difficult. However, the Emergency Center at Germantown is the ONLY location where an EDTA specimen is acceptable rather than fingertip smears.

Organisms are most likely to be detected if the smears are obtained immediately upon the onset of fever, or immediately before the fever is anticipated. In patients with a strong clinical history, but repeatedly negative results, multiple sampling throughout the fever may prove successful.

Platelets sitting on top of red blood cells may have the appearance of a ring form of malaria.

Precipitated stain may obscure malarial forms on the smear.

Identification to species should not be based solely on the examination of the thick smear preparation. Both thick and thin smears are required for a comprehensive blood parasite examination.

The patient's travel history may provide helpful information in the identification of malaria, *Babesia* species, and other blood parasites. Blood parasites are endemic to certain regions of the world; knowing what countries the patient has visited will aid in diagnosis.

The chart below can be used as a guide for diagnosis. It is not to be used as the primary diagnostic factor:

Blood Parasite	Endemic Area(s)
Plasmodium falciparum	Africa, Asia, Indian subcontinent, South America (Tropical areas worldwide)
Plasmodium vivax	Tropical and Temperate areas worldwide
Plasmodium malariae	Africa, Asia, Indian subcontinent, South America (Tropical areas worldwide)
Plasmodium ovale	West Africa, India, South America, some South Pacific Islands

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

N/A

14.2 Precision

N/A

14.3 Interfering Substances

N/A

14.4 Clinical Sensitivity/Specificity/Predictive Values

N/A

15. SAFETY

You, the employee, have direct responsibility to avoid injury and illness at work. Nearly all-harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Safety Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needle sticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.
- Warnings of other specific hazards are noted in this procedure. Please comply with the requirements to reduce your risk of injury."

Report all accidents and injuries to your supervisor or the Safety Officer.

16. RELATED DOCUMENTS

Hematology Slide Stainer Cytocentrifuge, Wescor Aerospray; Hematology procedure
Resulting Microbiology Direct Exams, Microbiology procedure
Malaria Smear, Phlebotomy procedure
Malaria History Form

17. REFERENCES

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18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP M028.005		
000	10/12/09	10.2.2	LIS upgrade to GUI system	A. Sears	R. Master
000	10/12/09	16	Added procedure for resulting	L. Barrett	R. Master
001	9/19/2011	3.1, 13	Added use of EDTA specimen at GEC	C. Reidenauer	R. Master
001	9/19/2011	4.2	Changed storage temperature for buffer	R. Master	R. Master
001	9/19/2011	8	Remove statement regarding pkg insert	L. Barrett	R. Master
001	9/19/2011	8.3	Added trypanosomes and microfilaria	R. Master	R. Master
001	9/19/2011	11.2	Update title to local terminology	L. Barrett	R. Master
002	11/19/12	9 10.2.1 10.2.2	Change report to “Parasitemia followed by the % infectivity”. Changed steps on how to report the % infectivity (English Text code first then free text the rate %)	M. Sabonis	R. Master

19. ADDENDA

None