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		Page 1 of 7	Date 09 January 2007

Title: EXAMINATION OF BLOOD SMEARS FOR MALARIA PARASITES

Author/Date: Anne Morrissey, December 2006

Approvals/Date:

John A. Crump, MB, ChB, Microbiology Laboratory Director

Anne Morrissey, MS, MT(ASCP)SM, Microbiology Lab Supervisor

This SOP has been read and understood by:

Name	Date
Signatures on original copy.	
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Document History:

Version Number	Reason for Changes	Date

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PURPOSE

Procedures for the examination of thick and thin Giemsa stained blood smears for the presence and identification of *Plasmodium* spp. (*P. falciparum, P. vivax, P. ovale, P. malariae*).

PRINCIPLE

Species specific identification of the *Plasmodium* spp. is clinically important for several reasons: *P. falciparum* has a high mortality rate and more likely to be drug resistant, eradication *of P. vivax* and *P. ovale* infections requires supplemental therapy. The thick blood film with lysed red blood cells (RBCs) is approximately 30 times more sensitive than the thin film for detecting the presence of malaria parasites (detecting about 20 parasites/μl**).** The thin film with intact RBCs is examined for speciation of malarial parasites allowing evaluation of the position of organisms within the cells, the size and shape of infected erythrocytes and presence of stippling within the cells.

SPECIMEN COLLECTION AND HANDLING

Refer to SOP MIC.040 PREPARATION AND STAINING OF SMEARS FOR BLOOD PARASITE EXAMINATION.

BLOOD PARASITE SMEAR RESULT LOG

Enter the following information on the Result Log:

Study ID#
Patient Name
KCMC Medical Record Number
Specimen Collection Date

QUALITY CONTROL

Positive Malaria Slide:

Frequency: Each new batch of stain prior to use and each day of patient testing.

Control: Thin smears prepared from blood from patient with high parasitemia (one parasite per 2-3 hpf), fixed in methanol and stored at -80^{o} C.

Acceptable Results:

1. Background should be clear and free from debris.
2. Nuclei of WBCs stain deep purple, cytoplasm pale purplish-blue.
3. RBCs stain pale pink.
4. Malaria parasite – blue rings, red chromatin dot.

Corrective Action for Unacceptable Results:

1. Restain new slide under supervision and reexamine.

2. If still unsatisfactory, prepare fresh stain and repeat QC.

Documentation:

1. Record QC results on GIEMSA STAIN QC SHEET.
2. Document all corrective actions for unacceptable results on QC Deviation form and submit to supervisor for review.
3. Review QC results monthly.

Slide Review:

1. As part of ongoing training and competency assessment of staff, all slides will be reviewed within 24 hours of initial reading with emphasis on suspected positive smears.
2. Discordant results between initial examiner and reviewer will be reviewed by the with technician and documented on Blood Parasite Smear Result Log.

EXAMINATION OF THICK SMEARS

1. Examine entire thick smear using low or high power objectives (10X, 25X) to detect large blood parasites such as microfilariae. Refer to SOP MIC.042 IDENTIFICATION OF NON-MALARIA BLOOD PARASITES if any are suspected. If other blood parasite is suspected have the slide reviewed immediately by Lab Supervisor.
2. Examine 100 fields using oil immersion objective (100X) for malaria parasite morphology. Select areas that are well stained and free of precipitate.
3. If no parasites are observed in 100 fields, continue examination until 300 fields have been observed.

EXAMINATION OF THIN SMEARS

1. Thin smears may be available earlier than thick smears which require longer drying time so can be examined first. If this is the case proceed as follows:
    a. Screen at low magnification (10X, 20X) for the presence of large blood parasites and proceed as instructed for thick smears.
    b. Examine 100 fields using oil immersion objective (100X). If no parasites are observed in 100 fields, continue examination until 300 fields have been observed
2. If thick smear has been examined and is negative, examination of the thin smear is not necessary.
3. If parasites are observed, quantify the number of asexual (trophozoites, schizonts) and sexual (gametocyte) forms (See Section – **QUANTIFYING PARASITES).**

Title: EXAMINATION OF BLOOD SMEARS FOR MALARIA PARASITES

IDENTIFICATION OF MALARIA PARASITES

The identification of the various *Plasmodium* species should be approached in an orderly fashion based on the observation of the following key morphologies:

- Ring forms and other asexual stages found in circulating in blood
- Appearance of the RBCs – enlarged, irregular or stippled
- Size and shape of developing and mature trophozoites
- Number of merozoites per schizont
- Morphology of gametocytes and internal pigment.

Refer to Table 1 and reference textbooks for examples of differentiating morphologies of *Plasmodium* spp. and infected erythrocytes.

Table 1. Differentiating features of *Plasmodium* spp.

Species	Stages found in peripheral blood	Appearance of RBC		Appearance of Parasite		
		Size	Stippling	Cytoplasm	Pigment in mature schizonts, gametocytes	No. of merozoites/ schizont
Plasmodium falciparum	Rings (mature) Gametocytes	Normal	None	Double dot in rings common Rings often small and delicate >1 ring /RBC	Black	6-32 (schizonts rarely seen in peripheral blood)
Plasmodium vivax	Trophozoites (all stages) Schizonts Gametocytes	Enlarged	Schuffners dots	Ring forms in all stages Ameboid trophs Light blue Large, circular gametocytes	Golden brown	12-24
Plasmodium ovale	Trophozoites (all stages) Schizonts Gametocytes	Enlarged, irregular shapes	Schuffners dots	Rounded compact trophs Dark to medium blue, usually dense	Dark brown	6-14
Plasmodium malariae	Trophozoites (all stages) Schizont Gametocytes Few rings usually seen –ring stage short.	Normal	None	Rounded compact trophs Dark blue dense cytoplasm Trophozoite band forms seen occasionally	Dark brown coarse	6-12

Title: EXAMINATION OF BLOOD SMEARS FOR MALARIA PARASITES

QUANTIFYING MALARIA PARASITES

The number and type of morphological forms observed (asexual –ring forms, trophozoites, schizonts vs. sexual – gametocytes) is an important diagnostic tool for appropriate treatment. They may indicate possible drug resistance and/or determine prognosis if serial specimens are examined after treatment is initiated.

Several different schemes for quantifying malaria parasites exist. In order to maintain consistency the procedure for quantifying parasites from Tanzanian patients will be the same as is conventionally used.

1. On the thick smear, using the 100x objective count the number of asexual forms (trophozoites, schizonts) seen per 200 WBCs.
2. If any sexual forms (gametocytes) are observed during the examination of the fields above, continue to examine fields containing 300 more WBCs and count the number per 500 WBCs.

PROCEDURAL NOTES

1. Care must be taken to avoid identifying superimposed platelets as malarial parasites. Superimposed platelets routinely exhibit a "HALO" whereas malarial parasites do not. It is also helpful to compare the suspected parasite to platelets that normally appear between the erythrocytes.

REFERENCE RANGE

Normal - No parasites found

RESULT REPORTING-MALARIA PARASITES

If a malarial parasite is seen but identification to species level is difficult, results should be reported: "*Plasmodium* sp. seen". If it is possible to determine that it is not *P. falciparum*, report "*Plasmodium* sp., not *P. falciparum*".

1. Enter test results BLOOD PARASITE SMEAR REPORT LOG.
2. Fill out a MICROBIOLOGY/IMMUNOLOGY RESULT FORM and ISAAC MISC TEST RESULTS DATA FORM and enter test results.
3. A positive slide for a blood parasite is considered a **Critical Result** and must be reported as soon as possible to physician taking care of the patient.
4. Document receipt of test information in Critical Results Log book and have physician sign the log book to acknowledge receipt of information.
5. Place negative patient reports in ward Lab Results box.

REFERENCES

MalariaGEN: Guidelines for Diagnostic Procedures for Blood Samples, Centers for Disease Control, Atlanta, GA.
Diagnostic Procedures for Blood Specimens for, Centers for Disease Control, Atlanta, GA, July 2003.

Rogers, W.O. 2003. *Plasmodium* and *Babesia*. In: Manual of Clinical Microbiology. 8th Ed. ASM Press, Washington, DC.

Centers for Disease Control, Division of Parasitic Diseases website: www.dpd.cdc.gov/DPDx

APPENDIX A - Blood Parasite Smear Results Log
APPENDIX B – Giemsa Stain QC Record
APPENDIX C – ISAAC Microbiology Test Report Form