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Section: Parasitology Manual	Subject Title: Protocol for Malaria Smears	
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PROTOCOL FOR MALARIA SMEARS

INTRODUCTION

Malaria is caused by infection with the *Plasmodium* parasite. Of the four species associated with human disease, *P. falciparum* is potentially the most dangerous. Recently there has been a continued marked increase on the number of cases of malaria in travelers and immigrants. There have been two recent fatalities. In addition individuals who appear relatively well can progress in 1-3 days to become critically ill and require intensive care. It is therefore essential to have rapid and accurate diagnosis of this parasite as well as access to expert clinical advice on the diagnosis and management of the disease. The key features which determine treatment are: 1) the species of malaria, and 2) the percentage of red blood cells (RBCs) that are infected (i.e. % parasitemia). Testing for malaria should be done on anyone who has a fever or a history of fever (within 72 hours) and who has traveled to or through a malaria endemic area (most of the tropics and subtropics) within 1 year (and especially within 3 months).

MALARIA SERVICE AND TURN-AROUND-TIME (TAT):

A joint malaria protocol exists between the Hematology Department, Toronto Medical Laboratories (policy #QHE00001.01) and the Toronto Medical Laboratories/Mt. Sinai Hospital, Department of Microbiology (policy # MI\PAR\10\v03). A separate tube of blood is to be collected for the Hematology and Microbiology Laboratories.

All malaria bloods are considered **STAT** and will only be performed and interpreted by properly qualified Medical Laboratory Technologists. Processing of blood samples for malaria is available 24 hours/day, 7 days/week. From 7:00 a.m. until 9:00 p.m., the Hematology Laboratory, 222 St. Patrick's Street (SPS), will screen all malaria samples and report results within 2 hours. After 9:00 p.m., all malaria bloods will be screened by either the Rapid Response Laboratory at Toronto General Hospital or by the Rapid Response Laboratory at Toronto Western Hospital, and the sample(s) will be forwarded to the Hematology Laboratory (SPS) with the first delivery (6:30 am) the following morning. Screening will include a thin film (BFR) examination for the presence of malaria parasites, calculation of the percentage of infected RBCs (% parasitemia) and performance of a dipstick test for the detection of *P. falciparum*. All blood samples, whether positive or negative, will be picked up daily by the Microbiology technician

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and/or technologist from the Hematology Laboratory. The Microbiology Laboratory will prepare thick and thin smears and speciate any malaria species seen. The Microbiology results will be reported under the test procedure “Plasmodium – Micro” within 18 hours of the initial Hematology Laboratory thin film and dipstick report, 7 days a week. It is the responsibility of the Microbiology technician and/or technologist receiving a blood sample for malaria to check with the Hematology Laboratory to ensure that they have also received a blood sample on the same patient. Processing of the blood sample in the Microbiology Laboratory should not be delayed because the Hematology Laboratory has not received a sample or has not yet reported their results.

ORDERING AND SAMPLE COLLECTION

A) University Health Network (UHN):

Two orders must be entered in MYSIS, one for “Plasmodium Screen – Hematology”, which is accessioned and resulted by the Hematology Laboratory and/or the Rapid Response Laboratories and one for “Plasmodium – Micro” which is accessioned and resulted by Microbiology/Parasitology. Therefore two lavender (EDTA) tubes of blood should be collected, one for each laboratory.

B) Scarborough Centenary Health Centre, a site of the Rouge Valley Health Centre:

Two separate orders must be entered in MEDITECH, one for “Malaria Screen” which goes to the Hematology Laboratory and one for “Malaria Thick and Thin Film” which goes to Microbiology/Parasitology. Therefore two lavender (EDTA) tubes of blood should be collected.

C) Other Hospitals:

Blood samples will be accepted from non-client hospitals, which will be billed by the Microbiology Department. Non-client hospital labs are asked to provide the original EDTA tube of blood and at least two unstained Thick and two unstained Thin Films. These samples are processed in the Microbiology/Parasitology Laboratory only following screening by the referring Hematology and/or Core Lab. The result will be phoned and/or faxed to the referring laboratory or physician within 18 hours. **A contact name and telephone number must be available for every malaria blood submitted.**

Specimen Rejection Criteria

Leaking, unlabeled, mislabeled, or broken tubes will not be accepted.

Hours of Service:

During regular operating hours of the Parasitology Laboratory, the Parasitology technician or technologist will pick up specimens and a copy of the screening report (if available) from the Hematology Laboratory. Samples that arrive after the lab is closed will be picked up first thing

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the following morning. Samples that arrive in the final hour of operation of the Parasitology Laboratory can be examined the next morning ***if the Parasitology technician or technologist can confirm that hematology will issue a report within 2 hours.*** If this will not be done, then it is the responsibility of the Microbiology/Parasitology technologist to process the blood and report the results.

On weekends and statutory holidays, a qualified Microbiology technologist will be on-call from **8:00 a.m. to 4:00 p.m.** When a malaria sample is received by Hematology, they will page the Microbiology technologist on-call by contacting Locating **(340-3155)** at Toronto General Hospital. The Microbiology technologist should call back to acknowledge receipt of the page, but will only return to the laboratory to process the malaria specimens once during the day. If no page is received, it is the responsibility of the Microbiology technologist on-call to call the Hematology Lab **at noon** to check if any malaria samples have to be reviewed. Should questions of a clinical nature arise, the physician should be directed to page the Microbiologist-on-call or the Infectious Diseases Service through Locating **(340-3155)** at Toronto General Hospital.

Follow-up smears during and after treatment:

These will be performed immediately upon request where there is a concern that the response to treatment is not adequate. In such a case, a clinical consultation must be obtained from Tropical Medicine or Infectious Diseases Service. Routine follow-up smears should be performed daily until discharge for *Plasmodium falciparum*, then at day 7 and day 28 after the start of treatment. For patients not admitted and for *Plasmodium vivax* infections, follow-up smears are performed on day 2 or day 3, day 7 and day 28.

Processing of specimens:

A thick and thin smear should be prepared and interpreted for all blood samples for malaria. If not already performed by the Hematology or Rapid Response laboratories, or the result is not available, then the blood sample should also be assayed for the presence of *Plasmodium falciparum* using a dipstick (antigen) test. The Makromed dipstick test is performed according to the supplied instructions. **A negative dipstick test does not rule out malaria due to other Plasmodium species.**

REPORTING PROTOCOL

Hematology will issue a preliminary report for the thin smear and dipstick result (policy #QHE0001.01). The Hematology result will print automatically in the virology report printer. It is the responsibility of the Parasitology/Microbiology technologist to check the printer for reports on a regular basis. For UHN patients *only*, the Hematology report will be viewable on-line in MYSIS. For non-UHN patients, or if the report fails to print, the report can be accessed by calling the Call Centre at 416 340-5898. If a hematology result is not available on a patient then perform a dipstick test (PHE14012.01) on the sample and report the result.

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A) If the results from Parasitology are consistent with the preliminary report from Hematology then report as follows:

- If the **thick and thin smears are negative**, report "NEGATIVE for *Plasmodium* by microscopy". Add the comment "Malaria cannot be ruled out as a diagnosis on the basis of a single result. If malaria remains as a diagnostic consideration three serial blood samples at 12-24 hour intervals should be submitted".
- If the **smear is positive** for *P. falciparum* report "POSITIVE for *P. falciparum* malaria by microscopy" and report the parasitemia and stages.
- If a **mixed infection is present** that includes *P. falciparum* report "POSITIVE for a mixed infection that includes *P. falciparum* and _____ malaria by microscopy" and report the presence of the additional species, parasitemia and stages.
- If the **dipstick performed in the Parasitology laboratory is positive** and the **smear is negative** report "POSITIVE for *P. falciparum* by HRP2 antigen detection assay, no malaria parasites seen on blood film". **If it is a follow-up blood** add the statement "Dipstick tests can continue to give positive results for up to 4 weeks after successful treatment." (Refer to **Discrepant Results** below)
- If the **smear is positive** for a species other than *P. falciparum* and the **dipstick is negative** report "POSITIVE for *P. _____* by microscopy"
- All results must be phoned to the appropriate ward or attending physician. All positive results for UHN patients must also be phoned to the Infectious Disease Service.
- All new positive Malaria smears for UHN patients will be phoned to the requesting physician, the Infectious Disease resident on-call, Dr. Jay Keystone and/or Dr. Kevin Kain.
- All results are to be signed out by a qualified technologist without queuing for review by the charge/senior technologist. A list of all Malaria testing is available on-line using the lab information system.

B) If the final report from Parasitology is discrepant with the preliminary report issued by Hematology, proceed as follows:

- Notify Hematology immediately of the discrepancy.
- Otherwise, follow the reporting procedure outlined above.

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Discrepant results:

Discrepant results may occur if:

- The individual has been successfully treated for *P. falciparum*. The HRP-2 antigen may continue to circulate for up to 28 days after the infection has been treated.
- The antigen detection test is known to cross react with rheumatoid factor and thereby may produce a false positive result if this is present in the sample.
- In rare circumstances, the HRP-2 protein produced by *P. falciparum* may not be recognized by the antigen detection assay. It is therefore possible that the assay will completely miss a significant parasitemia.
- If a mixed infection is present, the antigen assay will detect the presence of *P. falciparum*, however another species may be present in larger numbers.
- After successful treatment, the thick film smear may remain positive for a longer period of time as it is more sensitive than the thin film.

If further testing is required to resolve whether *Plasmodium* is present, consult Dr. Ian Crandall or Dr. Kevin Kain to discuss further testing.

NOTE: The presence of Leprosy, *Cryptosporidium*, *Cyclospora*, *Entamoeba histolytica*, *Giardia lamblia*, *Trichinella spiralis* and all species of malaria are reportable to the Medical Officer of Health by the next working day. For more information, please refer to the General Information Manual, page 34.