

# Comparison of Optimal Malarial Test with Light Microscopy for the diagnosis of Malaria

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### Abstract

**Objective:** To evaluate the efficacy of a parasite lactate dehydrogenase-based immunochromatographic antigen detection assay (optimal) against conventional light microscopy in the diagnosis of malaria at Military Hospital, Rawalpindi and Department of Pathology Army Medical College Rawalpindi from August to October 2002 in patients reporting sick with history suggestive of malaria.

**Methods:** The blood samples were collected from 215 patients reporting with symptoms suggestive of malaria. Thick and thin blood films were prepared, stained with Leishman's stain and examined by light microscopy for the presence of malarial parasites. Parasitaemia was estimated on all positive slides. All samples were tested for presence of malarial parasite by optimal dipstick method according to the manufacturer's instructions.

**Results:** A total of 215 cases were studied. Malarial parasites were visualized in 98 (45.5%) cases with light microscopy. Optimal test revealed 93 (43.2%) of these samples as positive. Microscopy showed 61 out of 98 positive cases to be *P. vivax* and 37 *P. falciparum*. The Optimal dipstick method revealed that 58 out of 93 were positive for *P. vivax* and 35 positive for *P. falciparum*. These results demonstrated that Optimal had sensitivities of 95% and 94.5% for *P. vivax* and *P. falciparum* respectively. It has 100% specificity for both malarial species, when compared to conventional microscopy.

**Conclusion:** Optimal test showed excellent correlation with microscopy in the diagnosis of *P. vivax* and *P. falciparum*. It is expensive but it has an advantage of being simple, rapid and effective test in the diagnosis of malaria especially where well trained microscopists are not available or work load is too high (JPMA 54:404;2004).

### Introduction

Malaria remains an important parasitic disease worldwide. Three to five hundred million people contract the disease annually. It is estimated to kill between 1.5-3.5

million people each year.<sup>1</sup> Prompt and accurate diagnosis is the key to effective disease management. Therefore it is one of the main interventions of the global malaria control strategy.<sup>2</sup>

Several approaches are available for the diagnosis of Malaria. Clinical diagnosis, still widely used could be only 50 % accurate.<sup>3</sup> Examination of thin and thick blood film under the light microscope is considered a gold standard in the diagnosis of malaria. It is a sensitive, informative, inexpensive but labor-intensive method, requiring at least 60 minutes from specimen collection to result.<sup>4</sup> Fluorescent microscopy is sensitive especially quantitative buffy coat examination. This method can screen quickly many samples but requires special equipment and training.<sup>5,6</sup> Malarial pigment can be screened by dark-field microscopy but sensitivity is low.<sup>7</sup> Polymerase chain reaction based molecular assays are the most sensitive but it cannot be employed for routine purposes.<sup>8</sup> Latest automated haematology analyzers can flag suspicious samples by detecting malarial pigment in the white blood cells but still requires confirmation by another method.<sup>9</sup>

Development of malarial antigen based rapid diagnostic tests (Rdts) for detection of malaria in the past few years might offer a valid alternative to microscopy.<sup>7</sup> These tests are based on the detection of antigens derived from malaria parasites in lysed blood, using immunochromatographic methods. Most frequently they employ a dipstick or test strip bearing monoclonal antibodies directed against the parasite antigens. These tests can be performed in 15-20 minutes.<sup>10,11</sup> Several commercial kits are available detecting two different malarial antigens. Histidine-rich protein2 (HRP-2) is a water soluble protein detected by ICT Malaria and parasite-F kits. These detect plasmodium falciparum only and the test is positive for some days even after successful treatment.<sup>12,13</sup> Another dipstick assay (Optimal) detects plasmodium specific lactate dehydrogenase (pLDH). It can separate plasmodium species by detecting antigenic differences between various p-LDH isoenzymes. The test quickly becomes negative with treatment and promises to be quite sensitive and specific.<sup>14</sup>

We evaluated the performance of Optimal by comparing its sensitivity and specificity with light microscopy in detecting malarial parasite in patients with presumptive clinical diagnosis of malaria.

## Patients and Methods

Two hundred and fifteen patients reporting sick from August 2002 to October 2002 in Military Hospital Rawalpindi with symptoms suggestive of malaria were included in the study. The clinical presumption of malaria was based on febrile illness of short duration associated with chills, rigors and body aches. Patients of all ages were included in the study. A detailed history and clinical examination was undertaken in all these subjects. Patients having any other obvious reason for fever and those who had

taken antimalarials were excluded from the study.

Three ml venous blood was collected in ethylenediamine tetra acetic-acid anticoagulated bottles from each patient. Thin and thick blood smears were prepared, stained with Leishman's stain and examined for malarial parasite by light microscopy using 100 × oil immersion. Parasite number was counted per 200 white blood cells in P. falciparum positive thick smears. Parasite density was expressed as number of parasite / ul. A blood smear (thin and thick) was considered negative if no parasite was seen after 10 minutes of search or examination of 100 high power fields of microscope.<sup>1,9</sup>

Same blood samples were also tested by Optimal dipstick method according to manufacture's (Flow inc. Portland, OR, USA) instructions. The immunochromatographic test detects the presence of pLDH antigen in lysed whole blood. pLDH is released from live malarial parasites and differentiation of plasmodium species is based on antigen differences between its isoforms. Aside from a control antibody reaction zone at the top of the test strip, the optimal dipstick contains two test lines or reaction zones. The first line encountered by the sample comprises of an antibody that is specific for P. falciparum pLDH. The second test line is composed of a pan specific pLDH monoclonal antibody that recognizes all other plasmodium species (P.vivax, P. malariae and P. ovale).

Two drops of reagent A (30 ul of colloid/ buffer solution) were added to the conjugate well and four drops of reagent B (8 ul of clearing solution) were added into the wash well provided on a configured well plate. Ten microlitres of blood was added to the conjugate well and mixed gently. Dipstick was placed vertically into the conjugate well and allowed to stand for 10 minutes. The dipstick was then transferred to wash well and left there until the bands became clearly visible within 5-10 minutes. The interpretation of results was performed immediately after completion of the clearing step as follows:

1. Positive - P. falciparum; one control band and two test bands.
2. Positive - P. vivax and other plasmodium species; one control band and one test band.
3. Negative - One control band at the top of the strip. Examples are shown in Figure.

## Results

Out of 215 blood samples 98 (45.5 %) were positive with microscopy, whereas 93 (43.2%) were positive with optimal. Out of 98 positive by microscopy, 61 were P. vivax and 37 P. falciparum. Out of 93 Optimal positive, 58 were P. vivax and 35 P. falciparum. These results demon-

positive, 58 were *P. vivax* and 35 *P. falciparum*. These results demonstrated that Optimal has sensitivities of 95% and 94.5% for *P. Vivax* and *P. falciparum* respectively, when compared to conventional microscopy. It has 100% specificity for both malarial species. Optimal dipstick method missed two cases of *P. falciparum* that were positive by microscopy and these were the cases who had a low parasitic density. The comparison of sensitivities of two methods in respect of *P.falciparum* density is shown in Table.

Two cases of mixed malarial infestation were observed on microscopy. In both these cases Optimal gave the result as *P. falciparum*. One case showed only gametocytes of *P. falciparum* and no ring forms were seen on microscopy. Optimal showed the case positive for *P. falciparum* infestation. It required minimum sixty minutes to deal with one blood sample by microscopy whereas with Optimal method, the results were available within 15-20 minutes.

## Discussion

The study compared the results of a new rapid malarial diagnostic method (Optimal) with traditional light microscopy. It was found that both the methods yielded comparable results. Optimal missed two cases of *P. falciparum* and three cases of *P. vivax* which were positive on microscopy. There could be two reasons for these false negative results. Firstly, Optimal is not sensitive below a parasitic index of 100 parasite/ul.<sup>15</sup> It is also reflected in our results as all the *P. falciparum* cases which were missed by Optimal had a parasitic density below 100 parasite/ul. Secondly, Optimal detects pLDH which is produced by living parasites. It is possible that some patients might have already taken antimalarials earlier and had not disclosed it.

For clinical use the sensitivity of the Optimal as currently configured should be adequate for malarial diagnosis. Majority of patients of *P. falciparum* infestation (as in this study 58 out of 61) usually have parasitic index greater than 100 parasite/ul. This is also true for *P. falciparum* infestation in non-endemic areas and in non-immune patients. Microscopy can pick malarial parasites even if the density is as low as 10-50 parasites/ul.<sup>9,15</sup> This sensitivity of microscopy is seen under study conditions where trained microscopists are scanning each blood smear for 10-20 minutes which does not seem practical in a busy hospital setting in routine. We are of the view that in routine hospital laboratory practice the sensitivity of Optimal should be comparable to microscopy or even more. Optimal does not have any subject bias whereas in microscopy the results are affected by the skill and workload of the microscopists.

Two cases of mixed malarial infestation were observed on microscopy. Optimal reported these cases as *P. falciparum* due to the configuration of the test. This

inevitably missed the other species, but as *P. falciparum* is the species which causes the most majority of clinical complications, the test format is not disadvantageous. One case in this study showed only the gametocytes of *P. falciparum* in the blood smear and no ring forms were seen. Optimal reported it as *P. falciparum* infestation as pLDH is secreted by gametocytes. These Optimal results can lead to unnecessary medication.

Several authors have previously reported trials of Optimal for malarial diagnosis from different countries with more or less similar results. Palmer et al<sup>16</sup> in Honduras has reported a sensitivity of 94% and specificity of 100% for *P. vivax* and sensitivity of 88% and specificity of 99% for *P. falciparum*. From hospital of tropical diseases UK, Moody et al<sup>17</sup> while conducting their study in sub-Saharan Africa, Asia and South America have reported a sensitivity of 95.3% and specificity of 100% for *P. falciparum* and sensitivity of 96% and a specificity of 100% for plasmodium vivax. John et al<sup>18</sup> obtained a sensitivity of 94% for *P. falciparum* and 98.2% for *P. vivax* in a trial in Southern India. In Gambia, Hunte-Cooke et al<sup>19</sup> reported a sensitivity of 91.3% and a specificity of 94% for *P. falciparum* with Optimal. A short study carried out at Combined Military Hospital Quetta by Ahmad et al<sup>20</sup> has reported a sensitivity and specificity of 100% for both species. Our results are comparable in sensitivity and specificity from most of the above mentioned studies carried out in different parts of the world. Therefore the results of this study further substantiates that Optimal is an effective and sensitive tool in the diagnosis of

Microscopy is the gold standard for diagnosing malaria. Although it is time consuming, as one test requires 60 minutes, but it is economical and accurate if performed by a patient and skilled technologist. In comparison, the Optimal test is simple more objective, requires no equipment, but is more expensive. However, the cost factor becomes less significant due to the reduced morbidity and hospital admissions.

## References

1. Makler MT, Palmer CJ, Ager AL. A review of practical techniques for the diagnosis of malaria. *Ann Trop Med Parasitol* 1998;4:419-33.
2. A global strategy for malaria control. Geneva, WHO 1993.
3. Chiodini PL. Non-microscopic methods for diagnosis of malaria. *Lancet* 1998;351:80-1.
4. Bain BJ, Chiodini PL, England J M, et al. The laboratory diagnosis of malaria. *Clin Lab Haemat* 1997;19:165-70.
5. Baird JK, Jones TR. Diagnosis of malaria in the field by fluorescence microscopy of QBC capillary tubes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1992;86:3-5.
6. Benito A, Roche J, Molina RA, et al. Application and evaluation of QBC malaria diagnosis in a holoendemic area. *Applied Parasitology* 1994;35:266-72..
7. Hanscheid T. Diagnosis of malaria: a review of alternatives to conventional microscopy. *Clin Lab Haematol* 1999;21:235-45.

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- Makler MT, Palmer CJ, Ager AL. A review of practical techniques for the diagnosis of malaria. *Ann Trop Med Parasitol* 1998;4:419-33.
- A global strategy for malaria control. Geneva, WHO 1993.
- Chiodini PL. Non-microscopic methods for diagnosis of malaria. *Lancet* 1998;351:80-1.
- Bain BJ, Chiodini PL, England J M, et al. The laboratory diagnosis of malaria. *Clin Lab Haemat* 1997;19:165-70.
- Baird JK, Jones TR. Diagnosis of malaria in the field by fluorescence microscopy of QBC capillary tubes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1992;86:3-5.
- Benito A, Roche J, Molina RA, et al. Application and evaluation of QBC malaria diagnosis in a holoendemic area. *Applied Parasitology* 1994;35:266-72.
- Hanscheid T. Diagnosis of malaria: a review of alternatives to conventional microscopy. *Clin Lab Haematol* 1999;21:235-45.
- Humar AC, Ohrt MA, Harrington D, et al. Parasite F test compared with the polymerase chain reaction and microscopy for the diagnosis of *Plasmodium falciparum* malaria in travelers. *J Trop Med Hyg* 1999;56:44-53.
- Moody AH, Chiodini PL. Methods for detection of blood parasites. *Clin Lab Haematol* 2000;22:189-202.
- Jelinek T, Grobusch M P, Schwenke S, et al. Sensitivity and specificity of dipstick tests for rapid diagnosis of malaria in non-immune travellers. *J Clin Microbiol* 1999;1:227-30.
- Garcia MS, Kirimoama D, Mariborough J, et al. Immunochromatographic test for malaria diagnosis. *Lancet* 1996;347:1549.
- Kilian AHD, Mughusu EB, Kabagambe G, et al. Comparison of two rapid HRP-2 based diagnostic tests for *Plasmodium falciparum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1997;91:666-7.
- Beadle C, Long GW, Weiss WR, et al. Diagnosis of malaria by detection of *Plasmodium falciparum* hrp-2 antigen with a rapid dipstick-capture assay. *Lancet* 1994;343:564-8.
- Piper R, Lebras J, Wentworth L, et al. Immunocapture diagnostic assays for malaria using *Plasmodium lactate dehydrogenase (p-LDH)*. *Am J Trop Med Hyg* 1999;60:109-18.
- Congpuong K, Bualombai P, Jitchamroen S, et al. Comparison of Optimal rapid test with routine microscopy examination of Giemsa-stained thick blood film for diagnosis of malaria. *Thailand J Med Assoc* 2001;84:357-63.
- Palmer CJ, Lindo F, Klaskala W, et al. Evaluation of the optimal test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* malaria. *J Clin Microbiol* 1998;36:203-6.
- Moody A, Hunt-Cooke A, Gabbet E, et al. Performance of the optimal malaria antigen capture dipstick for malaria diagnosis and treatment monitoring at the hospital for tropical diseases, London. *Br J Haematol* 2000;109:891-4.
- John SM, Sudarsanam A, Sitaram U, et al. Evaluation of optimal, a dipstick test for the diagnosis of malaria. *Ann Trop Med Parasitol* 1998;92:621-2.
- Hunt-Cook A, Chiodini PL, Doherty T, et al. Comparison of a lactate dehydrogenase-based immunochromatographic antigen detection assay (optimal), with microscopy for the detection of malaria in human blood samples. *Am J Trop Med Hyg* 1999;60:173-6.
- Ahmad SQ, Abbasi SA, Tariq MA, et al. Evaluation of *Plasmodium lactate dehydrogenase* based immunochromatographic kit for the diagnosis of malaria. *J Coll Physicians and Surgeons Pak* 2003;13:176-7.

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