PERFORMANCE OF THE OPTIMAL TEST FOR MALARIA DIAGNOSIS AMONG SUSPECTED MALARIA PATIENTS AT THE RURAL HEALTH CENTERS

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Abstract. The OptiMAL test detects both Plasmodium falciparum and P. vivax malaria infections. In this study, we evaluated the performance of the OptiMAL test at the Basic Health Units (BHUs) and the District Health Quarter (DHQ) Center in rural villages of Punjab, Pakistan that provide minimal health services. Two sets of blood specimens obtained from 930 suspected malaria patients attending these BHUs were tested at BHUs and the DHQ Center by microscopy and the OptiMAL test. At the BHUs, 231 (25%) of the patients were positive by microscopy and 278 (30%) patients tested positive by the OptiMAL test. At the DHQ Center, microscopic analysis of a second set of specimens from the same patients confirmed the malaria infection in 386 (42%) patients and the OptiMAL test result was positive in 300 (32%) patients. To determine the performance of OptiMAL test at the BHUs and the DHQ Center, all data were compared with microscopy results obtained at the DHQ Center. The OptiMAL test results for P. falciparum at the BHUs were comparable to those of the OptiMAL test at the DHQ Center. However, the sensitivity, positive predictive value (PPV), and negative predictive value (NPV) of the OptiMAL test were considerably lower for P. vivax infections than for P. falciparum infections, irrespective of whether the test was performed at the BHUs or at the DHQ Center (P. falciparum: sensitivity = 78–85%, PPV = 89–97%, NPV = 96–98%; P. vivax: sensitivity = 61–76%, PPV = 88–95%, NPV = 90–93%). The OptiMAL test also detected a number of false-positive and false-negative results at both the BHUs and the DHQ Center. The false-positive results ranged from 1% to 2%; however, the number of false-negative results was much higher (BHUs: P. falciparum = 22%, P. vivax = 39%; DHQ Center: P. falciparum = 15%, P. vivax = 24%). In conclusion, these results, when combined with other advantages of the OptiMAL test, suggest that this test can be used by relatively inexperienced persons to diagnose malaria infection in rural areas where facilities for microscopy are not available.

INTRODUCTION

In patients with malaria, a prompt and accurate diagnosis is the key to effective disease management. Microscopic examination of stained blood films remains the standard diagnostic method. However, correct interpretation of blood films requires considerable expertise that is not necessarily available at peripheral medical centers in non-endemic countries. Thus, availability of a simple and accurate test could greatly aid the diagnosis of malaria infection in remote areas where health facility coverage is low and the population is at high risk of contracting malaria.

Antigen-detection methods have recently been introduced for situations where reliable microscopy may not be available. In the case of infection with Plasmodium falciparum, these new rapid methods are based on detection of histidine-rich protein-2 (PIHRP-2; ICT Malaria Pf and ParaSight-F) or parasite lactate dehydrogenase (pLDH; OptiMAL). Species-specific pLDH isoforms have been used to develop a test for P. vivax (OptiMAL). Plasmodium vivax can also be detected by detection of antibodies against pan-malarial antigen in the absence of those against HRP-2 (ICT Malaria Pp/Pv). The sensitivity and specificity of each of these tests have been assessed in a range of clinical situations.

This study was conducted at the Basic Health Units (BHUs) in rural areas of Punjab, Pakistan where malaria infection is endemic. These BHUs form the tertiary health care centers and lack the services of an expert microscopy to confirm malaria infection. The study also investigated the operational aspect of this rapid non-microscopic test by health workers with minimal technical expertise in diagnosing malaria infection.

MATERIALS AND METHODS

Patients and study design. This study was conducted at the BHUs that form the tertiary clinics situated in rural areas of Punjab, Pakistan for the provision of basic health care services. A trained Lady Health Visitor (LHV) and a volunteer health worker (VHW), both of whom are not experts in microscopy, service the BHUs. Residents attend the BHUs located in their village. Three BHUs in central Punjab, Pakistan, where malaria infection is endemic, were selected to participate in this study. The research team provided appropriate training in the use of OptiMAL kits to LHVs and the VHW. The selection of the OptiMAL test in this study was based on performance of this test and our experience in handling this test at the Microbiology Unit, Faculty of Medicine, of the University of Kuwait.

Nine hundred thirty individuals aged 2–55 years of age who were attending the BHUs with a history of fever and possible malaria infection were included in the study. Individuals that have been treated for malaria in the previous four weeks were excluded from the study. For each individual, paired 0.5-mL peripheral blood samples were taken in a pre-heparinized Eppendorf tube (Eppendorf-Netheler-Hinz, GmbH, Hamburg, Germany). One set of samples was used to make blood films for microscopy and for the OptiMAL test at the BHUs, while the second sample was coded and sent to the District Health Quarter (DHQ) Center for OptiMAL testing and microscopic analysis. The research team also examined randomly selected Giemsa-stained blood smears and stained OptiMAL strips at the Microbiology Unit, Faculty of Medicine of the University of Kuwait. The microscopic analysis of Giemsa-stained smears and the OptiMAL tests at the BHUs and
to the DHQ Center were performed in double-blind manner. Informed consent to participate in the study was obtained from the participants and the local Ethical Committee reviewed and approved the study.

**Microscopy of Giemsa-stained blood films.** Thick and thin blood films were made immediately after blood collection and stained with 10% Giemsa for 10 minutes and analyzed by light microscopy. A minimum of 200 consecutive fields was counted in the thick blood film before classifying a slide as negative. Parasites in thick blood films were counted against 200–500 white blood cells. The parasite density was estimated assuming 8,000 white blood cells/μL of blood.1,16,17

**OptiMAL test.** The OptiMAL test (Flow, Inc., Portland, OR) was performed as previously reported following the manufacturer’s instructions.8,9 Briefly, one drop of whole blood was mixed with two drops of lysis buffer A, which disrupts the red blood cells and releases the pLDH. The specimens were then allowed to migrate to the top of the pLDH strip. After eight minutes, the strips were placed in washing buffer B, which clears the hemoglobin from the strip. Interpretation of the test results was performed immediately. A negative control sample taken from an individual who had not been exposed to malaria for three years, was included with each batch tested. In the pLDH assay, there are two diagnostic zones of reaction containing different antibodies. The first diagnostic zone contains a monospecific antibody that recognizes only *P. falciparum* if it is present. The second diagnostic zone contains a pan-specific antibody immediately above the first zone. This monoclonal antibody recognizes the pLDH isoforms of *P. vivax*. A third reaction zone containing a pan-specific monoclonal antibody is present at the top of the test strip and serves as a positive control for the assay. The test can be completed in 10–15 minutes.

**Statistical analysis.** Data was collected and analyzed using the SPSS (SPSS, Chicago, IL) statistical program. For sensitivity and specificity, the test kits were compared with the microscopic results of Giemsa-stained smears at the DHQ Center. Sensitivity was calculated as the proportion of positive test results obtained among samples containing malaria parasites by microscopy; specificity was calculated as the proportion of negative test results obtained among samples whose thick blood films were negative. Positive predictive values (PPVs) and negative predictive values (NPVs) were calculated as the proportion of true positive results among all positive reactors and as the proportion of true negative results among all negative reactors, respectively.

**Results**

Blood specimens were collected in duplicate from 930 suspected cases of malaria. All of these patients presented with a history of fever of 2–3 days duration and none of them had a history of antimalarial therapy during the last four weeks. One set of specimens was tested for malaria infection by microscopy and the OptiMAL test at the BHUs and the other set was tested at the DHQ Center. The results of microscopy of Giemsa-stained blood films and the OptiMAL test at both the BHUs and the DHQ Center are summarized in Table 1. At the BHUs, 231 (25%) of the patients were positive for malarial parasites by microscopy (92 for *P. falciparum*, 126 for *P. vivax*, and 13 for mixed infections of both of these parasites), while 278 (30%) patients tested positive by the OptiMAL test (115 for *P. falciparum*, 144 for *P. vivax*, and 19 for mixed infections). At the DHQ Center, microscopy of the second set of specimens from the same patients confirmed malaria infection in 386 (42%) patients (131 for *P. falciparum*, 206 for *P. vivax*, and 49 for mixed infections), while 300 patients tested positive by the OptiMAL test (114 for *P. falciparum*, 164 for *P. vivax*, and 22 for mixed infections). The performance of OptiMAL test in detecting malaria infection at the BHUs and the DHQ Center was compared with the microscopic results obtained at the DHQ Center. The staff at the BHUs detected malaria infection by microscopy in 60% of the patients (70% for *P. falciparum*, 61% for *P. vivax*, and 26% for mixed infections). However, when using the OptiMAL test, the same staff detected malaria infection in 72% of the patients (88% for *P. falciparum*, 70% for *P. vivax*, and 39% for mixed infections).

At the BHUs, the number of malaria cases confirmed by the OptiMAL test was significantly higher (P < 0.05) than that by microscopy. It is notable that the OptiMAL test results for *P. falciparum* at the BHUs were comparable with those of the OptiMAL test at the DHQ Center (Table 1). As expected, the OptiMAL test had a lower sensitivity for lower parasitemias when compared with microscopy (Table 1), but it showed better performance than microscopy at the BHUs even at lower parasitemias. A number of false-positive and false-negative results were detected by the OptiMAL test at the BHUs and the DHQ Center (Table 2). The OptiMAL test failed to detect infection in 109 patients, 80 (39%) with *P. vivax* and 29 (22%) with *P. falciparum*, that were positive by microscopy at the DHU Center (Table 2). Of particular concern, however, were high parasitemias in two patients that

| Table 1 | Summary of findings in 930 suspected malaria patients for microscopy and the OptiMAL test at the Basic Health Units and the District Health Quarter Center |
|---|---|---|---|
| | Basic Health Units | OptiMAL test | District Health quarter center |
| *Plasmodium falciparum* | Microscopy | 92 | 115 | 131 |
|  | OptiMAL test | 126 | 144 | 206 |
|  | Total positive | 231 | 278 | 386 |
|  | Total negative | 699 | 652 | 544 |
| Parasite density/μL | 699 | 652 | 544 |
| >500 | 23 | 36 | 72 |
| 500–5,000 | 167 | 198 | 266 |
| >5,000 | 41 | 45 | 48 |
| Total | 231 | 278 | 386 |

*Note:* *P. malariae* and *P. ovale* are not included.
were not detected by the OptiMAL test at the DHQ Center. The sensitivity and the NPV of the OptiMAL test were considerably lower for \textit{P. vivax} infections than for \textit{P. falciparum} infections, irrespective of whether the test was performed by the inexperienced staff at the BHUs or by the trained technical staff at the DHQ Center (\textit{P. falciparum}; sensitivity = 78–85%, PPV = 89–97%, NPV = 96–98%; \textit{P. vivax}; sensitivity = 61–76%, PPV = 88–95%, NPV = 90–93%, Table 3). A total of 100 randomly selected blood specimens were also tested at the Microbiology Unit, Faculty of Medicine, of the University of Kuwait. Both microscopy of Giemsa-stained blood smears and the OptiMAL test result for \textit{P. falciparum} and \textit{P. vivax} was comparable with the results obtained at the DHQ Center. However, 19 additional malaria cases (11 with \textit{P. falciparum} and 8 with \textit{P. vivax}) were detected by microscopy at the University of Kuwait that were negative by microscopy at the BHUs.

The procedural aspects of the OptiMAL test were rapid and easy to follow for the staff at the BHUs. The comparative performance, requirements, and technical specifications of the OptiMAL test and microscopy show that the OptiMAL test presents technical and operational advantages over microscopy (Table 4).

**DISCUSSION**

The recent development of easy, rapid, and accurate tests for the detection of malaria infection is highly commendable. One of the major goals of developing such tests was that these should be handled with ease and accuracy by relatively unskilled staff in the rural villages where microscopy may not be available for diagnosing malaria infection.\textsuperscript{1,2} This study was designed to investigate the performance of the OptiMAL test, relative to microscopy, at the BHUs in rural villages that have minimal health services. As such, the definitive diagnosis of malaria infection in such areas is severely compromised by the lack of microscopy. The choice to include the OptiMAL test in the study was based on its capacity to detect both \textit{P. falciparum} and \textit{P. vivax} infections and its performance in various epidemiologic settings.\textsuperscript{8,9} The study design was such to make a direct comparison of the performance of microscopy and the OptiMAL test when conducted by persons with different technical experience. The staffs at the BHUs tested one set of the duplicate specimens taken from the patients and experienced technical staff at DHQ Center tested the other set. Our data confirm that the sensitivity of microscopy for malarial parasites was very low (25%) at the BHUs compared with that (42%) at the DHQ Center. Compared with microscopy at the DHQ Center, the staff at the BHUs failed to detect at least 30% of the \textit{P. falciparum} and 39% of the \textit{P. vivax} malaria cases. The sensitivity was further lowered at parasitemias < 500/μL; 58% of the infections were not detected by microscopy at the BHUs. The staff at the DHQ Center was more experienced in making better blood slides. Therefore, it would be expected that the quality of the blood slides made by staff at the DHQ Center is higher than that of the BHUs, and that this difference may affect the reading of slides, resulting in a lower sensitivity of microscopy at the BHUs.

Compared with microscopy at the DHQ Center, the performance of the OptiMAL test was comparable to that at the BHUs and the DHQ Center (88% and 87%, respectively). The OptiMAL test was more sensitive in detecting \textit{P. falciparum} infections than \textit{P. vivax} infections. This trend, as well as the actual values for sensitivity, specificity, PPV, and NPV, was consistent with the results of similar studies.\textsuperscript{1,6,9–11,18}

**Table 2**

Comparative performance of the OptiMAL test in 930 suspected malaria patients at the Basic Health Units and District Health Quarter Center compared with microscopy of stained blood films at the District Health Quarter Center\textsuperscript{*}.

<table>
<thead>
<tr>
<th></th>
<th>Microscopy at District Health Quarter Center</th>
<th>OptiMAL test at Basic Health Units</th>
<th>OptiMAL test at District Health Quarter Center</th>
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<tbody>
<tr>
<td></td>
<td>Positive Negative</td>
<td>Positive Negative</td>
<td>Positive Negative</td>
</tr>
<tr>
<td>\textit{P. falciparum}</td>
<td>Positive (131) 29</td>
<td>102 29</td>
<td>111 20</td>
</tr>
<tr>
<td>Positive (799)</td>
<td>13 786</td>
<td>115 815</td>
<td>3 796</td>
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<tr>
<td>Total (930)</td>
<td></td>
<td></td>
<td>114 816</td>
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<tr>
<td>\textit{P. vivax}</td>
<td>Positive (206) 80</td>
<td>126 80</td>
<td>156 50</td>
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<tr>
<td>Positive (724)</td>
<td>18 706</td>
<td>144 786</td>
<td>8 716</td>
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<tr>
<td>Total (930)</td>
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<td>164 766</td>
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\textsuperscript{*} The data on mixed infections are not included.

\textsuperscript{1} Values are percentages. \textit{Pf} = \textit{Plasmodium falciparum}; \textit{Pv} = \textit{P. vivax}; PPV = positive predictive value; NPV = negative predictive value.

Sensitivity, specificity, and positive and negative predictive values were calculated using microscopy as the standard test as described in the Patients and Methods.
However, some studies have reported that the sensitivity of the OptiMAL test in detecting malaria infections was better for *P. vivax* than for *P. falciparum*.14,19

The OptiMAL test detected a number of false-positive and false-negative results at both the BHUs and the DHQ Center. False-positive results ranged between 1% and 2%; however, the number of false-negative results detected was much higher (BHUs: *P. falciparum* = 22%, *P. vivax* = 39%; DHQ Center: *P. falciparum* = 15%, *P. vivax* = 24%). False-positive OptiMAL results have also been previously reported.8,9,12 False-positive cases could occur if patients had a previous recent infection with malaria,6,20,21 or if patients had circulating rheumatoid factor.12,15,22 However, it is unlikely that these factors account for all such cases. It is more likely that most of these OptiMAL-positive cases were true positive results that were not detected by microscopy at the BHUs due to lack of microscopy. In addition, false-negative results by microscopy can occur if patients have undertaken self-medication prior to presentation. The practice of self-medication is relatively common in rural villages and is often not reported to the research team.

Several factors could have explained the low performance false negativity of the OptiMAL test. Previous studies have reported a significant decrease in the sensitivity of the OptiMAL test at parasitemias < 100/µL. In addition, it has been reported that pLDH activity decreased with antimalarial therapy.7,8 Thus, the unreported prior use of antimalarial medication is relatively common in rural villages and is often used by relatively inexperienced persons to diagnose malaria infection in rural areas where microscopic facilities are not available.

In conclusion, this study investigated the use of the non-microscopic, rapid OptiMAL test by relatively non-technical volunteer staffs at the BHUs in rural villages. The performance of the test was adequate at both the BHUs and the DHQ Center, and the results obtained correlated well with each other and with those obtained by microscopy at the DHQ Center.

These results, combined with other advantages of the OptiMAL test, such as availability of results in 10–15 minutes and relative simplicity compared with microscopy and other confirmatory tests, suggest that the OptiMAL test can be used by relatively inexperienced persons to diagnose malaria infection in rural areas where microscopic facilities are not available.

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