Diagnostic and Prognostic Utility of Rapid Strip (Optimal and Paracheck) Versus Conventional Smear Microscopy in Adult Patients of Acute, Uncomplicated *P. falciparum* Malaria in Mumbai, India

**NJ Gogtay**, SS Dalvi**, D Rajgor***, AR Chogle#, DR Karnad++, M Ramdas+, U Aigal##, NA Kshirsagar++

**Abstract**

Objectives: The present study compared the diagnostic and prognostic utility of two rapid tests (Paracheck and OptiMal) versus conventional smear microscopy.

Methods: Using two independent microscopists we carried out the three tests in 31 adult cases of smear positive, acute, uncomplicated *Plasmodium falciparum* malaria. All three tests were done pre-treatment, and on Days 8, 15 and 29.

Results: Compared to microscopy, the Paracheck had a sensitivity of 100%, while the OptiMal had a sensitivity of 83.7%. The lower sensitivity of OptiMal resulted from misidentification by both microscopists of 6/31 cases as *Plasmodium vivax*. As a follow up tool, the OptiMal was better than Paracheck, due to the earlier disappearance of the parasite LDH. Also in the Paracheck, between microscopists, there was a significant difference in reading the tests, on Days 8 and 15.

Conclusion: Our study reiterates, the continued utility of conventional smear microscopy.

**INTRODUCTION**

Malaria, a widely prevalent parasitic disease affects 500 million people each year and is associated with 2-5 million deaths. Rapid and early detection of the malarial parasite and early treatment of infection still remain the most important goals of disease management. The rapid diagnostic tests include the older ones based on the detection of the HRP-2 antigen secreted by *P. falciparum* alone, such as the ParaSight F test (Becton Dickinson, Meylan, France), ICT malaria Pf test to the more recent OptiMal (Flow, Inc., Portland, Oreg) which detects the parasite-specific lactate dehydrogenase (pLDH), a soluble glycolytic enzyme secreted by viable parasites. Beyond diagnosis, the logical utilization of these diagnostic tests is to monitor treatment outcome. Several authors have used the ParaSight F test for this purpose and found that, the persistence of HRP-2 for prolonged periods (7-28 days) in drug-sensitive patients limited its utility as a prognostic tool. Our own findings in Indian patients in both complicated and uncomplicated *P. falciparum* malaria substantiated this. The OptiMal has also been used to monitor treatment outcome by Moody et al., Srinivasan et al., and Palmer et al., and has been shown to be better than the HRP-2 tests, since the pLDH persists for only 7-10 days.

With introduction of OptiMal in India in early 2001 and the availability of a relatively less expensive HRP-2 detection test, the Paracheck (Orchid biomedical systems, Goa, India) and in the absence of published literature in the country comparing the two tests; we carried out a prospective study evaluating the utility of the two tests. The objectives were two-fold - test utility as initial diagnostic tools, and test utility in monitoring treatment outcome during follow up. The study was carried out in adult patients of acute, uncomplicated *P. falciparum* malaria in Mumbai, India and smear microscopy was taken as the gold standard for comparison.

**METHODS**

The protocol was approved by the institutions ethical...
completed follow up upto day 29.

Criteria for evaluable patients was taken as those who had the initial diagnosis or the day of treatment/follow up. Microscopists reading the peripheral smear and were unaware the rapid diagnostic tests were different from the peripheral smears, the microscopists (M1 and M2) reading issues in reading both the rapid diagnostic tests and the positives + false negatives. Since subjectivity is one of these tests was calculated as true positives divided by true negatives. The number of parasites was counted against 200 white cells by the actual white cell count. The reading was done by trained, and skilled microscopists with at least seven years of smear reading experience. A maximum of 300 thick film fields was read before a slide was declared negative. Both rapid diagnostic tests were performed exactly as per the manufacturers’ instructions. The OptiMal, Paracheck and OptiMal were read before a slide was declared negative. Both readers microscopists reported all tests negative. This difference between readers was statistically significant (McNemars test, P < 0.05, 95% CI 0.23 and 0.09). With OptiMal, both M1 and M2 reported 27/31 as being negative, and 3/31 as Plasmodium falciparum. Of the remaining 1/31, M1 reported this as negative, while M2 reported this as Plasmodium falciparum.

On day 8, 27/31 blood smears were negative and 4/31 were positive for sexual forms only. With the Paracheck, M1 diagnosed 31/31 as being positive, while M2 diagnosed only 26/31 as being positive. This difference between readers was statistically significant (McNemars test, P < 0.05, 95% CI 0.23 and 0.03). With OptiMal both readers microscopists reported all tests negative. On day 15, 29/31 blood smears were negative and 2/31 were positive for sexual forms only. For Paracheck, both M1 and M2 diagnosed 25/31 as being still positive and 2/31 being negative. However, of the remaining 4/31, M1 diagnosed them as being positive by Paracheck, while M2 reported them to be negative. This difference between readers was statistically significant (McNemars test, P < 0.05, 95% CI 0.23 and 0.03). With OptiMal both readers microscopists reported all tests negative. On day 29, 31/31 smears were negative. For Paracheck, both M1 and M2 diagnosed 17/31 as still being positive, and 10/31 as being negative. Of the remaining 4/31, M1 reported them as Paracheck positive, while M2 reported them as Paracheck negative. With the OptiMal, both M1 and M2 reported all cards as negative.

The above results are summarized in Table 1.

<table>
<thead>
<tr>
<th>Day</th>
<th>Microscopy</th>
<th>Paracheck</th>
<th>OptiMal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M1 31 0</td>
<td>M1 31 0</td>
<td>M1 25 6</td>
</tr>
<tr>
<td></td>
<td>M2 31 0</td>
<td>M2 31 0</td>
<td>M2 25 6</td>
</tr>
<tr>
<td>8</td>
<td>M1 4 27</td>
<td>M1 4 27</td>
<td>M1 0 3</td>
</tr>
<tr>
<td></td>
<td>M2 2 29</td>
<td>M2 2 29</td>
<td>M2 0 3</td>
</tr>
<tr>
<td>15</td>
<td>M1 2 29</td>
<td>M1 2 29</td>
<td>M1 0 3</td>
</tr>
<tr>
<td></td>
<td>M2 0 3</td>
<td>M2 0 3</td>
<td>M2 0 3</td>
</tr>
<tr>
<td>29</td>
<td>M1 0 3</td>
<td>M1 0 3</td>
<td>M1 0 3</td>
</tr>
<tr>
<td></td>
<td>M2 0 3</td>
<td>M2 0 3</td>
<td>M2 0 3</td>
</tr>
</tbody>
</table>

31 evaluable drug sensitive patients of acute, uncomplicated Plasmodium falciparum malaria evaluated the diagnostic and prognostic utility of two rapid diagnostic tests- the Paracheck (HRP-2) and the OptiMal (parasite specific LDH) versus conventional smear microscopy, using two independent microscopists. Adult

RESULTS

Thirty five consecutive Plasmodium falciparum malaria patients diagnosed on blood smear microscopy (31 male, 4 female) with age ranging from 16-45 years; baseline asexual parasitemia ranging from 32-64,000/µl; and baseline sexual parasitemia ranging from 80-7920/µl were enrolled. 4/35 patients were lost to follow up, and 31 were considered evaluable. All patients were clinically and parasitologically cured (negative blood smear upto day 29 for asexual forms) and none were seen with resistance/recrudescence. On day 1, 31/31 patients were positive by blood smear microscopy for Plasmodium falciparum, and 31/31 positive by Paracheck, by both M1 and M2 giving Paracheck a sensitivity of 100%. With OptiMal, both M1 and M2 reported 25/31 as Plasmodium falciparum, and 6/31 as Plasmodium vivax. Both M1 and M2 reported the same 6/31 as being Plasmodium falciparum. Thus versus smear microscopy, the sensitivity of the OptiMal for diagnosing Plasmodium falciparum was 83.7%.

DISCUSSION

The present study in 31 evaluable drug sensitive patients of acute, uncomplicated Plasmodium falciparum malaria...
patients were chosen with a view to having a homogenous population, and due to the lack of pediatric malaria data in the country. It was seen that versus microscopy, the Paracheck for initial diagnosis had a sensitivity of 100%, while the OptiMal had a sensitivity of 83.7%, with both microscopists. Follow up of these patients with all three tests for prognostic utility showed that there was greater concordance while reading the peripheral smear as against the rapid diagnostic tests.

The rapid diagnostic tests like the ParaSight F test, ICT-Pf test and the Paracheck test have all been evaluated as initial diagnostic tools by several authors. Proux et al have shown that the Paracheck test has a sensitivity of 92.3%, for initial diagnosis of *Plasmodium falciparum*. The sensitivity of 100% seen in the present study with Paracheck could be attributed to the high baseline parasitemia that is reflective of more severely ill patients seen in a tertiary referral centre.

For the OptiMal test, Hunt Cooke et al have shown a sensitivity of 91.3% for initial diagnosis, while Piper et al have shown a 100% sensitivity as compared to smear microscopy. However, Fryauff et al have shown that the concordance between OptiMal and smear microscopy was 81% and 78% with two independent readers. The results of our study with OptiMal are similar due to misidentification of 6/31 cases of *Plasmodium falciparum* as *P. vivax* for initial diagnosis and leading to a low sensitivity of 83.7%.

The need for rapid malaria diagnostic tests was mandated by the fact that microscopic examination of peripheral smears is labor intensive, requires considerable expertise and that the vast majority of malaria cases occur in areas that do not have access to laboratory or microscopy facilities. We used the Paracheck test in our study as against that ParaSight F test or the ICT test in view of it being relatively less expensive, than the other tests that detect HRP-2 (1.5 US $ for the Paracheck versus 2.5 US $ for the other HRP-2 based tests). One of the reasons postulated for lower sensitivity of the OptiMal test by Ibqbal et al, was low parasitemias i.e., < 100 parasites/µl leading to misidentification of species. However, in these six patients of smear positive *Plasmodium falciparum* in our study who were misdiagnosed by the OptiMal test as *vivax* (the same 6/31 being misdiagnosed by both M1 and M2) had asexual parasitemias between 2440-7920/µl. The OptiMal test utilizes a *P. falciparum* specific 17E4 antibody, and a pan specific 19G7 antibody. For *P. falciparum* as well as mixed *vivax* and *falciparum* infections, both antibody bands turn positive. For *P. vivax* infections, only the pan specific 19G7 band turns positive. We hypothesize that in these six patients, reduced 17E4 antigen production, due to a different geographic strain, could probably account for non-appearance of the band and thus misidentification.

In India, and in the city of Mumbai, *Plasmodium vivax* is the predominant species (accounts for 80% of the malaria cases in the city, and 65% of cases in the country). Misidentification of *P. falciparum* as *P. vivax* as was seen in this study with the OptiMal test, for initial diagnosis would lead to the assumption of *P. vivax* and treatment with chloroquine to which a significant percent of *P. falciparum* in tertiary referral centres is resistant. The Paracheck is a useful tool for initial diagnosis of *P. falciparum*, as against the OptiMal, but as expected as a follow up tool, the persistence of antigenemia leads to sustained positivity and limits its utility, as shown by us earlier with the ParaSight F test. For follow up, the OptiMal test represents a better option as shown by at least 87% negativity (M2) on day 8 and complete negativity reported by both microscopists on days 15 and 29. This is similar to the findings of Moody et al. For the rapid tests, it is likely that faint lines in cases of low antigenemia subsequent to treatment, probably lend subjectivity to readings. The small sample size in the study was due to limited resources available for purchase of the diagnostic test kits, and a larger study would help confirm or alter findings, particularly in the community setting with wide ranging age groups and parasitemias. However, our study definitely underscores the continued utility of peripheral smear microscopy as the gold standard for malaria diagnosis.

Acknowledgements

This project was carried out at our department under the Advanced Centre for Research in Clinical Pharmacology set up by the Indian Council of Medical Research. Part funding was also received from the Research Society of the BYL Nair Hospital.

REFERENCES


---

**Announcement**

**Dr. PJ Mehta Young Scientist Award**

Papers are invited from young research workers (below 35 years of age) who have done original research work in the field of hypertension and related subjects. These papers will be judged by a panel of referees. The finalists will be required to present their papers during the XII National Conference of Hypertension, on 18th-19th October, 2003 at Hotel Fortune Pandiyan, Madurai, Tamil Nadu.

From these will be selected the recipient of the **Dr. PJ Mehta Young Scientist Award**. The research worker who submits his paper must attach a certificate to indicate his date of birth. The finalists will be given 2nd Class A/c train fare to and from their hometown. Please send 5 copies of the full manuscript of the paper along with the abstract typed to **Dr. BR Bansode, Secretary General, HSI, Dr. Babasaheb Ambedkar Memorial Hospital, Room No. 101, Central Railway, Byculla, Mumbai 400 027.**

Last Date of Receipt of Manuscript: **15th August, 2003**

Sd/-

BR Bansode
Secretary General, HSI