

**ORIGINAL ARTICLE****PARACHECK-PF<sup>®</sup> TEST VERSUS MICROSCOPY IN THE DIAGNOSIS OF *FALCIPARUM* MALARIA IN ARBAMINCH ZURIA WOREDA OF SOUTH ETHIOPIA****Hussein Mohammed<sup>1</sup>, Moges Kassa<sup>1</sup>, Amha Kebede<sup>1</sup>, Tekola Endeshaw<sup>2</sup>****ABSTRACT**

**BACKGROUND:** Malaria is a major cause of morbidity and mortality in Ethiopia. Rapid diagnostic tests such as Paracheck Pf are the major tools for falciparum malaria diagnosis as an alternative to microscopy in peripheral health facilities. The objective of this study was to evaluate the sensitivity and specificity of Paracheck Pf against microscopy for diagnosis of *P.falciparum* infection and observe the persistence of the antigen for an elongated period.

**METHODS:** Cross sectional study was undertaken in Arbaminch Zuria at Shele health center from October 2008 to January 2009. Paracheck-Pf versus microscopy comparison was done in conjunction with an artemisinin-based combination therapy efficacy monitoring for a period of 28 days. Standard microscopic procedures were done by experienced laboratory technicians and paracheck-Pf was performed in accordance with the manufacturer's instruction.

**RESULTS:** out of 1293 examined blood films, 400(31%) were found to be malaria positive. Considering microscopy as the gold standard, paracheck-pf showed sensitivity of 94.1 % (95%CI: 89.9-98.3%) and specificity of 80.0% (95%CI: 67.6-92.4%). The positive and negative predictive values were 93.3 % (95%CI: 88.8-97.8%) and 82.1% (95%CI: 70-94.1%), respectively. Comparing microscopy results 98.7 % (79/80), 60% (48/80), 48.1% (37/77), and 44.6 % (33/74) were also found to be positive by paracheck-pf at days 7, 14, 21, and 28, respectively.

**CONCLUSION:** Paracheck Pf<sup>®</sup> has a comparable diagnostic performance in detecting *P. falciparum* infections through the persistence of frequent false positivity is a limitation. Thus, this diagnostic test is not appropriate for monitoring of treatment effect.

**KEYWORDS:** *P. falciparum*, Paracheck-Pf<sup>®</sup>, RDT, microscopy.

**INTRODUCTION**

The global burden of malaria is currently estimated at over a million deaths annually, most of those who die are children, who mainly live in sub-Saharan Africa (1, 2). Like other African countries, malaria is a major public health problem in Ethiopia, covering 75% of the total area of the country. Annually around 4-6 million clinical malaria cases are reported across all the health facilities in the country and the actual number of malaria cases is estimated to be as high as 10-15 million (3). *Plasmodium falciparum* and *Plasmodium vivax* are the dominant human

malaria parasites and account for about 60% and 40% of the cases, respectively (4).

Prompt and accurate diagnosis of malaria is important for effective case management and if implemented well would reduce mortality from this disease. Like other African countries, peripheral health facilities do not provide laboratory services in most of the remote areas of Ethiopia (5). In such areas, resource for malaria diagnosis is unavailable or very scarce. Thus, diagnosis of malaria is often made on the basis of major clinical signs and symptoms without laboratory confirmation. This may lead to unnecessary wastage of drugs and misdiagnosis of other non-malaria febrile illnesses (5,6).

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The standard method for diagnosis of malaria infection is the microscopic examination of Giemsa's stained thick and thin blood films. Microscopy is helpful to identify *Plasmodium* species and to determine the parasite density but it requires a skilled and well experienced technicians, equipment and fresh reagents. In addition, it is time consuming and is not available in peripheral health facilities such as health posts. However, rapid diagnostic tests (RDTs) have been recommended to minimize the problems in areas where microscopy is not available (6).

Malaria RDTs are lateral-flow immuno-chromatographic tests that detect specific antigens produced by malaria parasites (7, 8). They are commercially available, in kit form, with all necessary reagents and are rapid when compared to microscopy and also can be performed easily by any medical staff without of the need of laboratory technician. These tests are easy to perform and do not require electricity or sophisticated equipment. It has an additional advantage of providing a result in 15-20 minutes time. Therefore, RDTs are more feasible for the diagnosis of malaria by health workers in remote areas where microscopy services are not available.

Malaria antigens currently targeted by RDTs are histidine-rich protein-2 (HRP-2), and two plasmodium enzyme based detection assays: plasmodium lactate dehydrogenase (PLDH) and plasmodium aldolase (9). HRP-2 is a protein uniquely synthesized by *Plasmodium falciparum* and present in the blood stream of an infected individual. Paracheck Pf is a monospecific RDT detecting *P. falciparum* HRP-2 antigens in blood specimens. However, Paracheck Pf has a disadvantage of not differentiating viable antigens from dead parasite antigens. Since HRP-2 is expressed only by *P. falciparum*, this test will give negative results for other non-falciparum plasmodium species (10). RDTs performance is, however, dependent on the correct storage, usage and interpretation of results, and the quality of the particular test used.

A number of studies have been conducted on the diagnostic performance of RDTs in Ethiopia during the previous years. But there has not been sufficient information of the study of persistent HRP-2 antigenemia. Therefore; the purpose the present study was to compare the diagnostic

performance of Paracheck Pf against microscopy during treatment follow-up period.

## SUBJECTS AND METHODS

A cross-sectional study was conducted at Shele Health Center in Arbaminch Zuria woreda South Ethiopia, with a catchment population of about 47,044 inhabitants. The Shele health center is located about 532 km south of Addis Ababa, and has an altitude between 1200 m to 1250 m above sea level.

A total of 1293 patients suspected for malaria infection (i.e., fever or history of fever for the past 48 hours or an axillary temperature of  $>37^{\circ}\text{C}$ ) were selected for microscopy diagnosis of the parasite between October 2008 to January 2009. Out of the total suspected malaria cases 158 (12.2%) were elected based on the following criteria: antimalaria treatment was not taken in the previous two weeks; were aged between 1 and 20 years; subjects who met these criteria were made eligible for the study and were parallel tested with RDT and microscopy.

Blood specimens were collected for preparation of blood smears and Paracheck Pf test (Orchid Biomedical Services, India) from finger prick using sterile lancet by experienced medical laboratory technician. Thick and thin smears were prepared on the same slide and stained with 3 % Giemsa solution for 30 minutes after fixing the thin smear with methanol. Thick smears were examined at 1000X magnification under the microscope at the health center and considered negative when no parasites were detected after examination of 200 microscopic fields. When positive for parasites, *Plasmodium* species identification was done from the thin smear. Both sexual parasites and gametocytes were counted against 200–500 white blood cells (WBCs) and expressed as number of parasite per  $\mu\text{l}$  of blood, assuming an average of 8,000 WBCs/ $\mu\text{l}$  of blood (11).

RDT paracheck Pf<sup>®</sup> (Orchid, Biomedical system, Verna, Goa, India) was performed according to the manufacturer's instruction. All RDT devices were labeled with similar patient ID numbers to that of the blood film. Then the collected blood sample was transferred directly to the sample pad and 6 drops of clearing buffer was added. Finally the results were read after 15

minutes. Presence of band on both the control and test lines indicated a positive result for *P. falciparum* infection. The formation of only one band on control line indicated a negative result. If no band formation on the control line, the test was invalid. The RDT used in this study were manufactured in April, 2008 with an expiry date of March, 2010 and were handled at the temperature recommended by the manufacturer suggesting that they were in a supposedly good condition during the study period.

RDT and microscopy results were read by different individuals at the health centre, each blinded to the results of the other diagnostic technique. All blood films were re-read and checked for the second time by an experienced microscopist blinded to the initial microscopy and RDT results. The discrepant readings were resolved by a third reader that was considered as a final result.

Out of those 158 recruited patients, 80 (6.2%) were recruited patients in the age range, 1-20 years and who were positive for *P. falciparum* mono-infections were included in the follow-up study in accordance with a WHO guideline (12). They were tested at days 7, 14, 21, and 28 as well the rate of clearance of parasitemia was diagnosed with RDT and microscopy.

Data entry and analysis were done using SPSS for windows version 12.0. The sensitivity, specificity, predictive positive and negative values of Paracheck Pf were calculated using microscopy as the gold standard. Briefly, Sensitivity was calculated as the proportion of positive test results against true positives  $[TP / (TP + FN)]$ ; Specificity was calculated as a proportion of negative test results against true negatives  $[TN / (TN + FP)]$ ; Positive Predictive Value (PPV) calculated as a proportion of true positive results among all positively reacting samples  $[TP / (TP + FP)]$ ; Negative Predictive Value (NPV) was calculated as the proportion of true negative results among all negatively reacting samples,  $[TN / (TN + FN)]$ ; and accuracy as  $(TP + TN) / \text{number of all tests}$ , where TP = true positive, FN=false negative, TN=true negative, and FP= false positive.

The study had Ethical clearance from the Ethiopian Health and Nutrition Research Institute (EHNRI) Ethical Committee. Blood film collection was done after the patients or their parents agreed to participate in the study and signed the consent form. Individuals who refused to sign the consent form were not included in the study. Study participants whose microscopy results confirmed malaria infection were offered immediate treatment according to the national guidelines (8).

## RESULTS

Out of a total of 1293 (673 male and 620 female) subjects clinically suspected, and microscopically examined, 400 (31%) were positive for malaria infection out of which. 291(73%) for *P. falciparum* infections, and 109 (27%) for *P. vivax* infections. Among the positives, two individuals had gametocytes of *P. falciparum* besides the asexual parasites. The proportion of malaria infections was 207(30.8%) in males and 193(31.1%) in females without significant difference (Table 1).

Table1. Positivity Rate of Malaria species with microscopy by sex (N=1293) Shele Health Center, Arbaminch Zuria, October, 2008 - January, 2009.

Number Examined	Number Positive (%)
Male (n=673)	207(30.8)
Female (n=620)	193 (31.1)
Total (n=1293)	400(31)

Among 158 study subjects screened for *P. falciparum* infection with both RDT and microscopy, 111(70.2%) of the cases were found to be positive for *P. falciparum* with both methods (Table 2). The accuracy of RDT test compared to microscopy showed a sensitivity of 94.1 % ( 95% CI: 89.9-98.3%), a specificity of 80.0 % ( 95%CI: 67.6-92.4%) a positive predict value (PPV) of 93.3 % ( 95%CI: 88.8-97.8%) and negative predictive value (NPV) of 82.1 % ( 95%CI: 70.0-94.1%).

Table 2. Diagnostic performance of Paracheck Pf RDT and microscopy results (n= 158), Shele Health Center, Arbaminch Zuria, October, 2008- January, 2009.

RDT (Paracheck-Pf)	Blood slide microscopy ( <i>Plasmodium falciparum</i> )		
	Positive	Negative	Total
Positive	111	8	119
Negative	7	32	39
Total	118	40	158

Sensitivity =94.1 % (95%CI: 89.9-98.3)

Specificity=80.0% (95%CI: 67.6-92.4)

Positive predictive value=93.3 % (95%CI:88.8-97.8)

Negative predictive value=82.1% (95%CI: 70.0-94.1)

The parasite density (number of parasites / $\mu$ l of blood) of the patients enrolled was as follows: 1000-10000 (n=36), 10000-20000 (n=16), 20000-50000 (n=18) and 50000-100000 (n=4). The proportion of patients with low parasite density (1000-20000 parasite/  $\mu$ l) was smaller 28.8% (15/52) than the proportion of high parasite density (20000-100000 parasite/  $\mu$ l) with sample

rate of 81.8 % (18/22) in a post 28 days of diagnosis for PfHRP-2 positive antigen. In general the presence of circulating HRP-2 antigen was detected at days 7,14,21 and 28 after effective treatment and 98.7 % (79/80) , 60 % (48/80) 48.1 % (37/77) and 44.6% (33/74) of the examined patients were still positive by RDT , respectively (Table 3).

Table 3. Positivity rate of Paracheck Pf and Microscopy after effective treatment with Coartem, Shele Health Center, Arbaminch Zuria, October, 2008- January, 2009.

Follow up Days	RDT Positive	Microscopy Positive
Day7	79/80 (98.7 %)	0
Day14	48/80 (60%)	1/80 (1.3%)
Day21	37/77 (48.1%)	0
Day28	33/74 (44.6%)	1/74 (1.4%)

## DISCUSSION

Parasite-based routine malaria diagnosis is focused on detection of asexual parasite stage in the stained blood smears using microscopy or detection of parasite antigen using RDTs. The present study has compared the performance of Paracheck-Pf test against the gold standard microscopy among the febrile patients in conjunction with treatment duration. Out of the 158 patients examined with microscopy and RDT, 118 (74.7%) were positive for *P. falciparum* malaria by microscopy while 119 (75.3%) were positive by paracheck. Thus, this finding shows that the *P. falciparum* detection rate of paracheck was comparable to microscopy. In this study, the sensitivity of the Paracheck Pf<sup>®</sup> observed in detecting *P. falciparum* was relatively lower than recorded by previous studies in Democratic Congo and Central India (13,14). This might be explained that the levels of parasitaemia can be below the

detection threshold (15). In the present study, however, a relatively high 80% specificity was observed compared to the studies conducted in Democratic Congo (13) and Central India (14). This might be related to the rate of patients that had already been successfully treated with antimalarial drugs, which might decrease the false positivity rate. In general, the accuracy of RDTs for the diagnosis of malaria infections depends on the quality of the kit, storage temperature and humidity, and end users' performance (5). In this study the Paracheck Pf RDT used was in a well-controlled condition, had a longer shelf life, were kept in the temperature ordered by the manufacturer.

On the other hand, the low sensitivity of RDTs below the level of 100 parasites per  $\mu$ l compared to microscopy is one of the drawbacks of RDTs (5, 14) which is supported by the result of the present study that shows 5.9 % (7/118) positive with microscopy were negative by

Paracheck pf, with parasitemia below the RDT's threshold detection level.

In the present study, circulatory HRP-2 antigen was detected in 60% of treated patients on day 14, and in 44.6 % it was still present on day 28. The other similar study conducted elsewhere showed that 98.2% and 92.0% of patients still had HRP-2 antigenemia after treatment at days 14, and 28, respectively (13). This discrepancy may be related to differences in the specificity data between the two studies, where their data unlike ours showed a low rate of 52% specificity (13). Therefore, RDT targeting HRP-2 would not be appropriate diagnostic device for monitoring treatment response due to persistent antigenemia.

The duration of false positivity observed in this study with HRP-2 test has been correlated to higher parasite density on admission. Since secretion of the protein is proportional to parasite numbers (13), a higher parasite density on admission would require an extended period of time for HRP-2 to be cleared from blood. Similarly, results from this study showed, twenty-eight days after effective treatment, 28.8% (15/52) of the patients with low parasite density had HRP2 false-positive results, compared to 81.8% (18/22) of those with high parasite density. Although the mechanism of HRP-2 clearance is not known, but potential causes for the presence parachute test after treatment include persistent parasitemia below the detection limit of microscopy and delayed clearance of circulating antigen (16).

In conclusion, Paracheck Pf test would be of great use for *P.falciparum* screening in areas that do not provide microscopy service, but persistence of HRP-2 antigen in the blood stream after treatment can affect interpretation of RDTs results. Therefore, one needs to incorporate the result of RDT in context of clinical history related to malaria diagnosis, particularly for intense malaria transmission areas but not to monitor treatment.

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