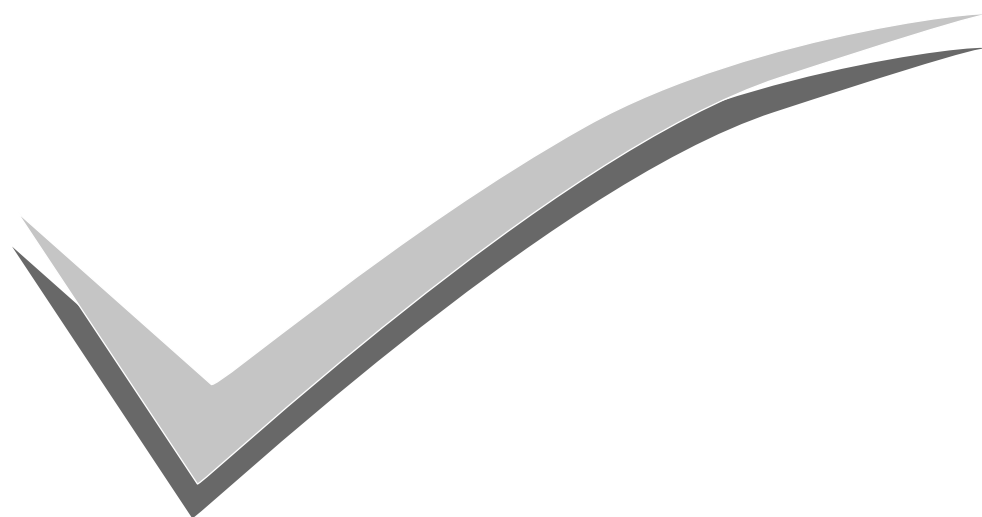




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S. No.	Names of Publications	Pg Nos
1.	Tanzania Journal of Health Research (2008), Vol. 10, No. 1	14-19
2.	JAPI • VOL. 51 • AUGUST 2003	762-765
3.	Pathogens and Global Health 2013 VOL. 107 NO. 2	69-77
4.	BMC Public Health 2012, 12:482	1-8
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Paracheck Pf® compared with microscopy for diagnosis of *Plasmodium falciparum* malaria among children in Tanga City, north-eastern Tanzania

M.L. KAMUGISHA^{1,2*}, H. MSANGENI¹, E. BEALE², E.K. MALECELA¹, J. AKIDA,
D.R.S ISHENGOMA¹ and M.M. LEMNGE¹

¹National Institute for Medical Research, Tanga Medical Research Centre, P.O. Box 5004, Tanga, Tanzania

²Morehouse College, Atlanta, Georgia, United States

Abstract: Malaria is a major public health problem particularly in rural Sub-Saharan Africa. In most urban areas, malaria transmission intensity is low thus monitoring trends using reliable tools is crucial to provide vital information for future management of the disease. Rapid diagnostic tests (RDT) such as Paracheck Pf® are now increasingly adopted for *Plasmodium falciparum* malaria diagnosis and are advantageous and cost effective alternative to microscopy. This cross sectional survey was carried out during June 2005 to determine the prevalence of malaria in an urban setting and compare microscopy diagnosis versus Paracheck Pf® for detecting *Plasmodium falciparum*. Blood samples from a total of 301 children (<10 years) attending outpatient clinic at Makorora Health Centre, in Tanga, Tanzania were examined for the presence of malaria. Twenty-nine (9.6%) of the children were positive to malaria by microscopy while 30 (10.0%) were positive by Paracheck® test. Three out of 30 positive cases detected by Paracheck® were negative by microscopy; thus considered to be false positive results. For the 271 Paracheck Pf® negative cases, 2 were positive by microscopy; yielding 2 false negative results. Paracheck Pf® sensitivity and specificity were 93.1% and 98.9%, respectively. *P. falciparum* was the only malarial species observed among the 29 microscopy positive cases. The prevalence of anaemia among the children was 53.16%. These findings indicate a low prevalence of malaria in Tanga City and that Paracheck Pf® can be an effective tool for malaria diagnosis

Key words: malaria, rapid diagnostic test, microscopy, anaemia, urban, Tanzania

Introduction

Malaria is a worldwide public health problem that continues to challenge researchers and health professionals alike. The disease affects approximately 300-400 million people (6-8% of the global population) and causes 1-2 million deaths every year (Snow *et al.* 2006;). It accounts for 10% of all hospital admissions and 20-30% of all doctors' visits annually, worldwide (WHO, 2004).

Proper management of malaria cases within the first 24 hours of onset is considered to be the best way to reduce its morbidity and mortality (Malimbo *et al.*, 2006; Hopkins *et al.*, 2007). This would be adequately achieved if most of the patients had access to laboratory facilities. Like in many countries in Africa (Petti *et al.*, 2006), in most areas of Tanzania, even where health facilities are available, most of them do not provide laboratory services. In these areas, treatment of malaria and other febrile conditions is usually based on clinical symptoms and guidelines such as the Integrated Management of Childhood Illness (IMCI) (Amstrong *et al.*, 2004). Yet the use of such guidelines without laboratory confirmation has lead to high levels of over-diagnosis and misuse of drugs (Reyburn *et al.*, 2004, 2007).

Microscopy is the golden standard diagnostic tool for malaria diagnosis in many areas of sub-Saharan Africa. However, use of microscopy requires well trained personnel and capital investment in terms of equipment (Petti *et al.*, 2006). Moreover, in cases where immediate diagnosis is necessary, microscopy is a drawback due to slightly time consuming methods involved in slide preparation and reading. In light of these, rapid diagnostic tests (RDTs) have been recommended for use in recent years (Petti *et al.*, 2006). These tools are easy to apply and provide immediate results even at rural setting (Mboera *et al.*, 2006a). Of the available RDTs, Paracheck Pf® has been proven advantageous due to its high reliability and low cost as compared to other RDTs (Proux *et al.*, 2001). Paracheck Pf® specifically detects *P. falciparum* histidine rich protein-2 (*Pf*HRP- 2) in whole blood specimens. Only a few studies have assessed the performance of Paracheck Pf® dipstick in detecting malaria parasites in different levels of endemicity in Tanzania (Mboera *et al.*, 2006a; Reyburn *et al.*, 2007), and most of them involved rural population.

In most urban areas of African countries malaria transmission intensity is low (Robert *et al.*, 2003). However, with the rapid growth of towns and cities which does not match with the infrastructure, there

* Correspondence: M.L. Kamugisha; Email: mkamugisha@tanga.mimcom.net

is concern of the expansion of malaria into the urban areas. In Tanzania, only a few published studies have been carried out on urban malaria in Dar es Salaam, Dodoma and Iringa (Yamagata, 1996; Wang *et al.*, 2006; Mboera *et al.*, 2006b). Thus, more information on the actual burden of urban malaria in different geographical areas of the country is urgently required to accurately determine its trend and provide necessary data for planning interventions. Thus, this study was conducted to determine the level of malaria burden using Paracheck Pf® dipstick and microscopy among children clinically diagnosed with malaria and attending a healthcare facility in Tanga City, Tanzania.

Materials and Methods

Study site

The study was conducted at Makorora Health Centre in Tanga City in north-eastern Tanzania. The City lies at about 5.17°, 5.33°S and 38.17°, 38.33°E along the Indian Ocean. The area receives two seasons of rainfall; short rains during October – November and long rains in March–May, with relative humidity of about 100%. Temperature ranges between 27°C and 32°C. Tanga City covers an area of about 600km² and has an estimated human population of 248,696. Malaria accounts for over 50% of all out-patient visits and admissions and malaria-specific deaths are estimated to be about 31% of all reported deaths (MoH, 2006).

Study design and data collection

A cross-sectional study was conducted for six weeks beginning the second week of June 2005. It involved a simple random cluster sample of 301 children aged ≤10 years attending Outpatient department (OPD) at the Health Centre. All the children had history and symptoms suggestive of malaria and were requested by the attending clinicians to be tested for malaria. The study was explained to children parents or caretakers and an oral consent was obtained before enrolment in the study. Following examination, results were communicated to the caretakers and Health Centre clinicians on duty.

Finger prick blood specimens were collected from each participating child. The collected specimens were used for preparation of blood smears and Paracheck Pf® dipstick test (Orchid Biomedical Services, India). Thick and thin smears were prepared on the same slide and stained with 10% Giemsa solution for 30 minutes after fixing the thin smear with methanol. The blood smears were carefully examined by skilled microscopists using an oil immersion lens. Smears were deemed

negative if there was no evidence of any parasites observed after reading 200 high power fields. In the event of the presence of parasites, the thick smear was used to determine the number of parasites present, while the thin smear was used to determine malaria species composition. Parasitaemia was specifically measured for asexual forms per 200 white blood cells (WBC) and gametocytes were counted per 500 WBC.

For detecting malaria parasites using Paracheck Pf®, fresh blood sample was transferred directly to the sample pad and results were read after 15 minutes as recommended by the manufacturers. Presence of both the control and test lines indicated a positive result. A negative result was indicated by the appearance of the control line alone. The result was considered invalid where both the control and test lines did not appear in which case the test was repeated. Axillary temperature was measured with a digital thermometer. Using the same prick, a sample of blood was collected for haemoglobin (Hb) concentration determination using Haemocue machine. Anaemia was considered when Hb level was < 11g/dl.

Statistical analysis

Data were entered in Epi Info (Centers for Disease Control & Prevention, Atlanta, GA, USA) and thereafter transferred, and analysed using STATA Version 8 for Windows. In order to investigate whether there was relationship between malaria status and other risk factors, Shapiro-Wilk test was used to test normality of continuous variables (age, haemoglobin level and measured body temperature). Chi-square test was done to determine whether there was an association between malaria status and other categorical variables, while ANOVA was used to determine the mean levels of each continuous variable in respect to other categorical variables under study interest. The effect modifier of the potential confounder value (age) with other risk factors, was also assessed. Akaike Information Criteria (AIC) method of comparing deviance of the two models that were nested was used, and the model with the least AIC value was selected as the best model. Hosmer-Lemeshow goodness-of-fit test was carried to assess the adequacy of logistic regression model obtained. The significance of the test was considered if p-value was ≤ 0.05.

The sensitivity, specificity, predictive positive and negative values of Paracheck Pf® were calculated using microscopy as the gold standard. Sensitivity was measured based on the number of true positive malaria cases identified correctly by Paracheck Pf® divided by the total number of those diagnosed as positive malaria cases by microscopy *plus* false negative

results. Specificity was measured based on the number of true negative malaria cases identified correctly by Paracheck Pf® divided by the total number of those diagnosed as negative malaria cases by microscopy plus false positive results. Positive Predictive Value (PPV) was measured based on the number of true positive malaria cases identified correctly by Paracheck Pf® divided by the total number of true positive cases and false positive cases obtained from the infected and non-infected cases. Negative Predictive Value (NPV) was measured based on the number of true negative malaria cases identified correctly by Paracheck Pf® divided by the total number of true negative cases and false negative cases obtained from the infected and the non-infected cases.

Results

A total of 301 children were involved in the study and were divided into three age groups: <1 year, 1-4 years, and ≥5 years. Most of the children (51.1%) were in the age group of 1-5 years and majority (54%) were males (Table 1). The mean and median age of the children was 2.54 and 1.7 years, respectively. Twenty-nine percent of the children were febrile (with temperature above 37.5°C). Eighty nine percent of parents/guardians reported using bednets out of whom 52.2% were using insecticide-impregnated nets.

Of the examined children, 29 (9.6%) were positive for malaria by microscopy while 30 (10%) were

positive by Paracheck Pf®. *Plasmodium falciparum* was the only malarial species observed among the 29 microscopy positive cases. Children aged 1-5 years comprised 58.6% of those diagnosed with malaria. Geometric mean parasite density of *P. falciparum* asexual forms (calculated by multiplying parasite count/200WBC) was highest in infants and lowest in children ≥5 years age group (Table 1). Geometric mean parasite density was not significantly different between age groups ($F = 1.49$, $P = 0.24$) and sex of the patients ($F = 1.46$, $P = 0.23$). Seven children (2.3%) had gametocytes (detected by microscopy). Two patients with gametocytes were microscopically negative but positive to Paracheck Pf® while other two patients had gametocytes but were negative for both microscopy and Paracheck Pf®.

The overall mean haemoglobin level was 10.92g/dl (95%CI 10.70 -11.14) and ranged from 4.70-16.00g/dl. The prevalence of anaemia was 53.16% and children less than 5 years of age accounted for 60.32% of all anaemia cases. Over 72% of the children diagnosed with malaria were also anaemic and anaemia was significantly associated with malaria ($P = 0.029$, $\chi^2=4.8$). When the effect of malaria status was controlled, a five-fold increase in age was associated with decrease in anaemia by 93.85% (95% CI: 60.68 - 99.06, $P = 0.004$). The risk of anaemia among children under five years decreased by 36% with an increase in age.

Table 1: Malaria parasite prevalence, density, haemoglobin level and anaemia by age group and sex at Makorora, Tanga

Age group/Sex	Pf +ve (microscopy)	GMPD*	Mean Temp.(95%:CI)	Mean Hb in g/dl (95%CI)	Anaemia (%)
Infants (n=98)	6.12	24322.38	37.47 (37.29 - 37.67)	10.35 (10.02-10.68)	68.37
1-4 yrs (n=154)	11.04	10879.34	37.14 (36.97 - 37.30)	10.83 (10.53 - 11.12)	55.19
5+yrs (n=49)	12.24	3164.79	37.12 (36.85 - 37.41)	12.33 (11.75 - 12.91)	16.31
Female (n=138)	10.87	15653.70	37.17 (37.0 - 37.34)	10.96 (10.69 - 11.23)	58.39
Male (n=163)	8.59	6126.62	37.31 (37.16 -37.46)	10.87 (10.51 - 11.22)	61.11

Key: Pf +ve = prevalence of *Plasmodium falciparum*, GMPD = Geometric mean parasite density; Temp.= temperature

Six variables were observed to fit well in predicting malaria status for the study children. These included age of the children, febrile status, being anaemic, and having symptoms of vomiting, abdominal pain and diarrhoea (Table 2). The risk of being a posi-

the standard, Paracheck® had a sensitivity of 93.1% and specificity of 98.9% with positive and negative predictive values of 93.3% and 99.2%, respectively which are similar to recent findings from other areas of Tanzania (Mboera *et al.*, 2006a). Taking into account

Table 2: Adjusted Odds ratio by age and symptoms related to the risk of malaria

Variable	Adjusted Odds Ratio	95% Conf. Interval	P-Value
Age	1.31	1.07 – 1.60	0.009
Febrile	4.17	1.73– 10.03	0.011
Anaemia	4.48	1.54 – 13.07	0.006
Vomiting	3.47	1.43 – 8.39	0.006
Abdominal Pain	1.63	0.48 – 5.50	0.432
Diarrhoea	0.40	0.16 – 1.15	0.207

tive case was observed to be 4.17 times more likely among febrile patients, and was also 4.48 times among anaemic patients (adjusted by other factors) (Table 2). This model indicated a predictive probability ability of model with these six variable to be 0.77 (95% CI: 0.66 – 0.88).

the fact that all the cases enrolled in our study were clinically diagnosed as malaria cases with intention to treat, it means that 90% of the patients would have received courses of antimalarials despite having no malaria parasites in their blood. Such overdiagnosis has been reported in northern Tanzania by Reyburn

Table 3: Sensitivity, specificity, positive and negative predictive value (PPV, NPV) of ParacheckPf®

Variable	Percentage	95% Confidence interval
Sensitivity	93.1	90.4 - 96.2
Specificity	98.9	98.2 - 100.0
PPV	93.3	90.4 - 96.2
NPV	99.2	98.2 - 100.0

Among the 30 positive cases detected by Paracheck Pf®, three were found to be negative by microscopy; yielding three false positive results (sensitivity = 93.1%). Also, among the 271 negative cases observed by ParacheckPf®, two cases were detected as positive by microscopy; yielding two false negative results (specificity = 98.2%) (Table 3).

Discussion

In general, the results from this study show that the performance of Paracheck® was comparable to microscopy. The prevalence of malaria by both microscopy and Paracheck® was similar. Only a small proportion of the children had measured fever (Temperature $\geq 37.5^\circ\text{C}$), indicating that most likely the children had received antimalarials/antipyretics prior to attending the health facility. Using microscopy as

et al. (2004) who found that only about half of the patients treated for malaria had negative results by microscopy.

The results of this study have shown that Paracheck Pf® is both a sensitive and specific test for *P. falciparum*. However, the test failed to detect three cases, which were positive and two cases, which were negative by microscopy, respectively. Too low levels of parasitaemia are likely to be reasons for the false negative results (Mboera *et al.*, 2006a). The false positive cases are likely to have been of patients who took antimalarials a few days prior to seeking care from the health facility.

Anaemia was prevalent among children attending the healthcare facility. Similar findings have been reported elsewhere in the country. For instance, recently, in a community-based study in southern Tanzania,

Schellenberg *et al.* (2003) reported higher prevalence of anaemia (87%) among children under five years of age. In our study, the risk of anaemia was observed to be about 5 times more likely in children with positive blood slides for malaria parasites than those without malaria parasites. Studies have already shown that malaria is an important cause of anaemia in endemic countries (Robert *et al.*, 2003). Therefore it is likely that most of the anaemia cases observed in our study were malaria related.

In conclusion, Paracheck Pf® is comparable to microscopy in detecting malaria in clinical cases. Malaria prevalence among children in Tanga City is low. Similarly, recent studies in Dar es Salaam (Wang *et al.* 2006) Dodoma and Iringa in Tanzania (Mboera *et al.*, 2006b) have shown low malaria prevalence among children living in urban areas. The low malaria prevalence observed in this study could be attributed to high mosquito net coverage as shown by the response of the caretakers. It could also be due to availability of few breeding sites for malaria mosquitoes. However, basic healthcare delivery systems providing early diagnosis and treatment and preventive actions such as the promotion of insecticide-treated mosquito nets for the rapidly growing numbers of the urban population needs to be promoted.

Acknowledgements

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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 the (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.¹ 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.^{2,3} The OptiMal has also been used to monitor treatment outcome by Moody *et al*,⁴ Srinivasan *et al*,⁵ and Palmer *et al*,⁶ 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

*Senior Lecturer, **Associate Professor, ***Senior Research Fellow, +Lab Technician, ++Professor and Head, Department of Pharmacology; +++Professor, Department of Medicine; Seth GS Medical College and KEM Hospital, Parel, Mumbai - 400 012. #Honorary Physician, ##Medical Superintendent, Kasturba Hospital for Infectious Diseases, Sane Guruji Marg, Mumbai - 400 011, India. Received : 22.6.2002; Accepted : 30.6.2003

committee, and written, informed consent was obtained from all participating subjects. Patients presenting to malaria outpatient department with symptoms and signs suggestive of malaria were screened by peripheral smear examination for the presence of malarial parasites. Consecutive patients, who satisfied inclusion criteria, were enrolled into the study. The inclusions were - age \geq 16 years, smear positive for *P. falciparum*, presentation of uncomplicated malaria, no history of having received any anti-malarial in the preceding two weeks, and willing to comply with protocol requirements. Exclusions were age less than 16 years, symptoms and signs suggestive of complicated malaria, pregnancy or lactation and mixed infections including *P. vivax*. Day 1 was taken as the first day of the study, and drug administration. All patients were hospitalized in the Clinical Pharmacology Infectious Diseases ward. They uniformly received treatment with quinine (30 mg/kg/d for seven days) and doxycycline 100 mg/d for seven days. Primaquine 45 mg single dose was given on Day 8, for its gametocytocidal action, which was also the day of discharge.

On day 1, prior to treatment, blood was collected by venipuncture for baseline peripheral smear, OptiMal, and Paracheck. The smears were all read within three hours, using both the Jaswant Singh Bhattacharya field stain as well as the Giemsa stain, and thick and thin smears were both read. The number of parasites was counted against 200 white cells and the parasite count obtained by multiplying this number 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 peripheral smear were repeated again on days 8, 15 and 29. Drug treatment for recrudescence (if any) was recorded on the case record form by the research assistant. Sensitivity of these tests was calculated as true positives divided by true positives + false negatives. Since subjectivity is one of the issues in reading both the rapid diagnostic tests and the peripheral smears, the microscopists (M1 and M2) reading the rapid diagnostic tests were different from the microscopists reading the peripheral smear and were unaware of the initial diagnosis or the day of treatment/follow up. Criteria for evaluable patients was taken as those who had completed follow up upto day 29.

RESULTS

Thirty five consecutive *P. 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/ μ l; and baseline sexual parasitemia ranging from 80-7920/ μ l 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 *P. vivax*. Both M1 and M2 reported the same 6/31 as being *P. vivax*. Thus versus smear microscopy, the sensitivity of the OptiMal for diagnosing *Plasmodium falciparum* was 83.7%.

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 0.09). With OptiMal, both M1 and M2 reported 27/31 as being negative, and 3/31 as *P. vivax*. Of the remaining 1/31, M1 reported this as negative, while M2 reported this as *Plasmodium falciparum*.

On day 15, 29/31 blood smears were negative and 2/31 were positive for sexual forms only. With 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 as 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 1 : Smear positive cases of *Plasmodium falciparum* malaria (n=31)

	Day 1		Day 8		Day 15		Day 29		
	+	-	+	-	+	-	+	-	
Microscopy									
M1	31	0	4*	27	2*	29	0	31	
M2	31	0	4*	27	2*	29	0	31	
Paracheck									
M1	31	0	31	0	29	2	21	10	
M2	31	0	26	5	25	6	17	14	
OptiMal									
	Pf	Pv	—	Pf	Pv	—	Pf	Pv	—
M1	25	6	0	0	3	28	0	0	31
M2	25	6	0	1	3	27	0	0	31

M1 - Microscopist 1; M2 - Microscopist 2; * Gametocytes only; Pf - *Plasmodium falciparum*; Pv - *Plasmodium vivax*; + positive; - negative

DISCUSSION

The present study in 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

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*⁷ 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 Cooke⁸ have shown a sensitivity of 91.3% for initial diagnosis, while Piper *et al* have shown a 100% sensitivity as compared to smear microscopy.⁹ However, Fryauff *et al* have shown that the concordance between OptiMal and smear microscopy was 81% and 78% with two independent readers.¹⁰ 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 or lowered sensitivity of the OptiMal test by Iqbal *et al*,¹¹ 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.¹² 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*.⁴ 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.

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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

Evaluation of Paracheck-Pf™ rapid malaria diagnostic test for the diagnosis of malaria among HIV-positive patients in Ibadan, south-western Nigeria

C. O. Falade^{1,2}, B. Adesina-Adewole³, H. O. Dada-Adegbola⁴, I. O. Ajayi⁵, J. O. Akinyemi⁵, O. G. Ademowo^{1,2}, I. F. Adewole^{3,6}, P. Kanki⁶

¹Department of Pharmacology & Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria,

²Institute for Advanced Medical Research & Training, College of Medicine, University of Ibadan, Ibadan, Nigeria,

³Department of Obstetrics & Gynaecology, College of Medicine, University of Ibadan, Ibadan, Nigeria, ⁴Medical Microbiology Department, College of Medicine, University of Ibadan, Ibadan, Nigeria, ⁵Department of Epidemiology, Medical Statistics, & Environmental Health, College of Medicine, University of Ibadan, Ibadan, Nigeria, ⁶APIN-Plus (PEPFAR) Project, College of Medicine, University of Ibadan, Ibadan, Nigeria

Febrile illnesses occur frequently among HIV positive patients and these are often treated presumptively as malaria in endemic areas. Parasite-based diagnosis of malaria will eliminate unnecessary treatment, reduce drug–drug interactions and the chances for the emergence of drug resistant *Plasmodium*.

We evaluated finger prick blood samples from 387 people living with HIV (PLWHIV) and suspected of having malaria by expert microscopy and Paracheck-Pf™ – a histidine-rich protein-II based malaria rapid diagnostic test. The study was conducted at the PEPFAR supported AIDS Prevention Initiative in Nigeria (APIN) Clinic of the University College Hospital Ibadan, southwest Nigeria. Outcome parameters were prevalence of malaria parasitemia, sensitivity and specificity of Paracheck-Pf as well as the positive and negative predictive values for Paracheck-Pf using microscopy of Giemsa-stained blood film as gold standard.

Malaria parasites were detected in 19.1% (74/387) of enrollees by microscopy and 19.3% (74/383) by Paracheck-Pf. Geometric mean parasite density was 501/μl (range 39–749 202/μl). Sensitivity and specificity of Paracheck-Pf at all parasite densities were 55.4% and 89.3% while corresponding figures at parasite densities ≥200/μl were 90.9% and 90.3%. Sensitivity and specificity at parasite densities ≥500/μl was 97.6% and 90.3%. Positive and negative predictive values for parasite density ≥200/μl were 55.4% and 98.7%, respectively.

Paracheck-pf was found to be a useful malaria diagnostic tool at parasite densities ≥200/μl facilitating appropriate clinical management.

Keywords: Adult, HIV, Malaria, Paracheck-RDT

Background

Human Immunodeficiency Virus (HIV) infection remains a global public health problem with over 34 million people living with HIV in 2010 and about 2.7 million newly acquired/diagnosed cases annually.¹ The bulk of the burden of HIV is in sub-Saharan Africa where malaria is endemic. The immune suppression that accompanies HIV naturally increases the susceptibility of HIV +ve persons to malaria.^{2,3} Not only do HIV positive persons have more frequent attacks of malaria,

the infection appears to be more severe in them.⁴⁻⁷

In malaria endemic areas, febrile illnesses are often assumed to be due to malaria and are usually treated as such without recourse to laboratory confirmation. Microscopy of Giemsa-stained blood smear, which remains the gold standard, is laborious and requires electricity to power the microscope and trained personnel, all of which are often not available in malaria endemic areas. Malaria has no pathognomonic signs or symptoms and presumptive treatment of malaria often leads to over-diagnosis of malaria and unnecessary treatment with antimalarial drugs.⁸ This practice carries with it numerous other problems, especially in HIV +ve persons, who are prone to many other infections such as

Correspondence to: Catherine O. Falade, Department of Pharmacology & Therapeutics, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria. Email: lillyfunke@yahoo.com

bacterial, mycobacterial, viral, fungal and other parasitic infections^{9–11} which could present with fever. Apart from the delay in appropriate diagnosis of non-malaria febrile illness and consequent progression of the disease process, unnecessary treatment with antimalarial drugs increases the already high pill burden of HIV patients who are on antiretroviral and/or opportunistic infection prophylaxis,¹² with the attendant risk of drug–drug interaction. Poor drug compliance (secondary to high pill burden) and emergence of drug resistant plasmodial or retroviral strains are some other possible risks.

These challenges underscore the need for accurate, inexpensive, simple to use, and rapid malaria diagnostic tests. Histidine-rich protein-II (HRP-II) based malaria rapid diagnostic tests (RDTs) have been recommended as good options.¹³ Histidine-rich protein-II based RDTs are more robust in that they can withstand the temperature fluctuations of tropical malaria endemic regions better than the enzyme-based RDTs, parasite lactate dehydrogenase (pLDH) and aldolase, which mandatorily require air-conditioned storage^{14,15} conditions that cannot be guaranteed in sub-Saharan Africa where the bulk of the malaria burden is.

Although Paracheck-PfTM has already been shown to be an effective tool for malaria diagnosis in many populations of children and adults in previous studies^{16–19} we nonetheless believe that it is important to evaluate its sensitivity and specificity among HIV positive persons especially in an area of high malaria transmission. In addition, some malaria RDTs have been shown to detect sub-microscopic infections.^{20,21} We report here the performance of Paracheck-Pf, an HRP-II based malaria RDT (Paracheck-Pf RDT), in the detection of *Plasmodium falciparum* in capillary blood samples of HIV +ve patients suspected of having malaria at the adult ARV out-patient clinic of the University College Hospital, Ibadan, south-western Nigeria, where malaria is endemic. This study was triggered by the routine prescription of anti-malarial drugs for patients attending the clinic which is often based on presumptive diagnosis.

Subject and Methods

Study site

The study was conducted at the HARVARD partnered President's Emergency Plan for AIDS Relief (PEPFAR) funded APIN adult ARV out-patient clinic, University College Hospital, Ibadan in south-western Nigeria. Ibadan lies at about 7°23'16" latitude and 3°53'47" longitude coordinates, with precipitation ranging from 24 to 178 mm/month and temperatures of 21.5–34.8°C in average conditions.²² Ibadan is located 160 km from the Atlantic coast and has an average elevation of 200 m. The human population of Ibadan is estimated to be about four

million. Malaria transmission is hyper-endemic in Nigeria, occurring throughout the year with a major peak during the rainy season months of May to October and a lower peak in the dry season months of November to April.²³ The prevalence of HIV seropositivity in Nigeria is 3.9% [UNGASS (2010), 'UNGASS Country Progress Report: Nigeria'].

Nigeria changed its malaria treatment guidelines in January 2005 in line with the World Health Organization recommendation from chloroquine to artemisinin-based combination therapy (ACT) with a preference for artemether–lumefantrine or artesunate–amodiaquine in that order.²⁴ The standard of care for malaria at the PEPFAR clinic of the University College Hospital is ACT. Both artemether–lumefantrine and artesunate–amodiaquine are available on prescription at no cost to patients who receive care at the PEPFAR clinic. However, antimalarial drugs ranging from chloroquine to ACTs are available as over-the-counter drugs in private drug stores in Nigeria. The PEPFAR clinic in University College Hospital is the main facility in the city of Ibadan where persons living with HIV (PLWHIV) receive care for HIV infection and other health needs. The facility runs an out-patient clinic five days a week and in-patient services seven days a week. There are 10 medical officers dedicated to the clinic and a large pool of specialists within the hospital to draw on for care. Services rendered include walk-in clinics, confirmatory western blot testing for HIV, high quality counseling unit, diagnostic and follow up viral load measurement, CD4 T-cell counts, and drug pick-ups.

Baseline insecticide-treated net (ITN) usage in south west Nigeria is 1–20.1% in pregnant women^{25,26} and 1.7–26.6% among the under-five-year-olds.^{27,28} Participants in this study had received ITNs whenever the Federal Ministry of Health made ITN available. We estimate ITN ownership to be about 40% among patients attending the PEPFAR clinic at the University College Hospital Ibadan where this study was conducted. Participants also receive cotrimoxazole prophylaxis if their T-cell lymphocyte count was <350 cells/mm³.

The University of Ibadan/University College Hospital Institutional Review Committee provided ethical approval for the study. A signed informed consent was also obtained from each study participant before enrollment.

Study design and study procedures

Clinical evaluation

In a prospective study, 387 PLWHIV who were registered and received care at the PEPFAR clinic of the University College Hospital were enrolled between October 2009 and September 2010. Convenience

sampling method was used in a prospective, descriptive study design in which every consecutive patient referred to the study team by attending physicians, if their symptoms were considered suggestive of malaria, were enrolled. Patients were enrolled if they had symptoms suggestive of acute uncomplicated malaria, were 18 years and above, and provided a written, informed consent. Patients who refused to provide informed consent or had clinical features of severe malaria were exempted. Following enrollment, an interviewer-administered questionnaire was used to obtain information on patient's socio-economic and demographic background, history of arthritis, medication history especially antimalarial therapy within two weeks of enrollment, and history of blood transfusion within the same time frame. Presenting symptoms and signs of current illness were recorded. Also recorded were CD4 counts and viral load within six months of enrollment into the study as well as the use of opportunistic infection prophylaxis. Clinical measurements included weight, pulse rate, and temperature of each study participant. Thereafter, thick blood smears were prepared from a finger prick for microscopy. Blood was also obtained from the same finger prick for Paracheck-Pf RDT testing and hematocrit evaluation.

Preparation of blood smears and microscopic examination

Thick blood smears were prepared from a finger prick aseptically. Blood smears were subsequently dried and stained using standard procedures. Prepared blood smears were then examined by two experienced microscopists using a light microscope at $\times 1000$ magnification for the presence and quantification of malaria parasites. The microscopists who were blinded to the results of Paracheck-Pf assay screened each smear independently, with the mean of the two counts per patient recorded as that enrollee's parasite count. Discordant readings were resolved by one of the senior investigators (COF). The definitive count of asexual parasites was calculated assuming a total white cell count of $8000/\text{mm}^3$. A blood smear was only declared free of parasites after examining 100 high power fields. Qualitative results of malaria microscopy was made available within three hours of enrollment and all smear positive patients received a six-dose regimen of artemether-lumefantrine, which is the standard of care in Nigeria.

Parasite detection using Paracheck-Pf and determination of hematocrit

Paracheck-Pf [Orchid Biomedical Systems, Goa, India] was used for detection of *P. falciparum* according to the manufacturer's instructions. Staining of both the control and test bands (irrespective of the intensity of staining) was taken as a positive result. Staining of only the control band was considered indicative of a negative

result whereas the test was considered invalid if the internal control band was not stained.

Capillary tubes were filled up to the mark with blood from the same finger prick used for preparation of blood smears and Paracheck-Pf test. The capillary samples were spun in a Hawksley™ micro-hematocrit centrifuge and read using a Hawksley reader.

Data analysis

All patient information and test results were entered into a computer and analysed using SPSS Version 15 (SPSS Inc. Chicago, IL, USA). Frequency tables were generated for relevant variables. Descriptive statistics such as means \pm standard deviations were used to summarize quantitative variables while categorical variables were summarized with proportions. The chi-squared test was used to investigate associations between two qualitative variables. Analysis of variance was used to compare the mean values of more than two groups. All analyses were done at the 5% level of significance. Outcome parameters were prevalence of malaria parasitemia, sensitivity, and specificity of Paracheck-Pf as well as the positive and negative predictive values for Paracheck-Pf. With the corresponding results of the microscopy taken as the 'gold standard', the sensitivity and specificity of Paracheck-Pf were calculated as $\text{TP}/(\text{TP} + \text{FN})$ and $\text{TN}/(\text{TN} + \text{FP})$, respectively. Positive predictive values (PPV) were calculated as $\text{TP}/(\text{TP} + \text{FP})$ and negative predictive values (NPV) as $\text{TN}/(\text{TN} + \text{FN})$, where TP = True positive, TN = True negative, FP = false positive and FN = False negative.

Results

Demographic characteristics

Three hundred and eighty-seven HIV positive adults with clinical features suspected to be malaria were enrolled at the PEPFAR clinic at the University College Hospital, Ibadan, Nigeria between October 2009 and September 2010. The ages of the study participants ranged from 18–70 years with a mean of $36.7 \text{ years} \pm 9.5$. Three hundred and twenty-two of the 386 (83.2%) enrollees who provided their ages were aged 45 years or less. A large majority (320; 82.7%) of the study participants were females, of which 52 (16.3%) were pregnant. The population of patients attending the PEPFAR clinic at the University College Hospital was about 16 000 with a 33% male, 67% female distribution at the time of the study. Almost 8000 patients were receiving ART in the study center at the time of the study. About two-thirds (246/387; 65.1%) of the study participants had received at least secondary school education while 326/387 (84.2%) were gainfully employed. Enquiries into the marital status of the study participants revealed that 73.5% (275/374) were married. Further details of the demographic characteristics of enrollees are shown in Table 1.

Clinical features of study participants

One hundred and forty-five of 387 (38%) participants had CD4 count ≤ 250 cells/mm³, 267/354 (69%) were on antiretroviral therapy while 174/387 (45.7%) were on daily cotrimoxazole prophylaxis. Thirty-three (8.5%) study participants were not on cotrimoxazole or antiretroviral therapy. Fever or a history of fever (71.6%) within 24 hours of enrollment was the most common presenting complaint among study participants. The four next most frequent presenting complaints among enrollees were headache (58.9%), anorexia (50.1%), aches and pains (49.9%), and chills and rigors (38.8%). Presenting symptoms occurring in more than 5% of the study participants are shown in Table 2. About half (191/378; 50.5%) of the study participants had an axillary temperature of 37.5°C or above while 52.7% (197/374) were anemic (hematocrit <33%).

About a third (124/387; 32.0%) of the study participants reported having taken antimalarial drugs

in the two weeks preceding enrollment. Seventeen (13.7%) of them had taken more than one course/type of antimalarial drugs. Sulfadoxine–pyrimethamine was the most frequently used antimalarial drug (55/124; 44.4%) followed by chloroquine 28 (22.6%), and various ACTs [artemether–lumefantrine (17), artesunate–amodiaquine (7) and others (4)]. Twenty-six (21%) study participants who took antimalarial drugs had a history of artemisinin monotherapy use while eight (6.5%) had taken amodiaquine monotherapy. Only four of the 55 patients who had a history of sulfadoxine–pyrimethamine use were pregnant.

Results of malaria screening by microscopy

Malaria parasite was detected in 19.1% (74/387) of enrolled patients by microscopy of Giemsa-stained thick blood smear. The parasite density ranged from 39/μl to 749 206/μl with a geometric mean parasite density of 501/μl. Thirty-nine of 74 patients (52.7%)

Table 1 Socio-demographic characteristics of HIV +ve patients suspected of having malaria enrolled into the Paracheck-Pf in the diagnosis of malaria

Characteristics	Number (%)
Sex (N = 387)	
Males	67 (17.3)
Females	320 (82.7)
Level of education (N = 378)	
None	39 (10.3)
Primary/Qur'anic	93 (24.6)
Secondary	142 (37.6)
Post-secondary	104 (27.5)
Occupation (N = 377)	
Student/unemployed/retired	51 (13.5)
Petty trader	113 (30.0)
Primary school teacher/junior civil servant/artisan/transporter	125 (33.2)
High school teacher/middle level civil servant/middle business person	70 (18.6)
Major business person/professional	18 (4.8)
Marital status (N = 374)	
Single	49 (13.1)
Married – monogamy	192 (51.3)
Married – polygamy	83 (22.2)
Separated	15 (3.9)
Lives with partner but not married	3 (0.8)
Divorced	6 (1.6)
Widowed	26 (7.0)

Table 2 Presenting symptoms occurring in over 5% of HIV +ve patients suspected of having malaria enrolled and result of malaria microscopy

Presenting symptoms	Number (%) 387 (100)	MP +ve	MP –ve
Fever or a history of fever	277 (71.6)	58 (20.9)	219 (79.1)
Headache	228 (58.9)	44 (19.3)	180 (80.7)
Loss of appetite (anorexia)	194 (50.1)	40 (20.6)	154 (79.4)
Aches and pains	193 (49.9)	43 (22.2)	150 (77.3)
Chills and rigors	150 (38.8)	33 (22.0)	117 (78.0)
Cough	139 (35.9)	23 (16.5)	116 (83.5)
Sleeplessness	125 (32.3)	24 (19.2)	101 (80.8)
Abdominal pains	109 (28.2)	20 (18.3)	89 (81.7)
Vomiting	94 (24.3)	23 (24.5)	71 (75.5)
Irritability	59 (15.2)	12 (20.3)	47 (79.7)
Body weakness/tiredness	43 (11.1)	7 (16.3)	36 (83.7)
Catarrh (rhinorrhea)	33 (8.5)	3 (9.1)	30 (90.9)
Dizziness	23 (5.9)	8 (34.8)	15 (65.2)

MP = Malaria parasite.

and 46 (62.2%) of the 74 patients with patent parasitemia had parasite densities $<200/\mu\text{l}$ and $<500/\mu\text{l}$, respectively, while 18/74 (24.3%) of them recorded parasite densities of 5000/ μl and above. Although malaria parasitemia was more prevalent among female participants (20% vs 14.9%), the difference was not statistically significant ($P = 0.396$). The prevalence of malaria parasitemia was also not significantly affected by pregnancy status among the women, educational status, or occupation. None of the presenting symptoms were positively correlated with malaria parasitemia be it at all parasite density, $\geq 200/\mu\text{l}$ or $\geq 500/\mu\text{l}$. There was no correlation between a positive history of antimalarial drug use within two weeks of enrollment and the result of malaria microscopy (Table 3). Malaria parasitemia was significantly less prevalent among patients receiving antiretroviral drugs but not Cotrimoxazole (Table 3). Patients whose CD4 counts were ≥ 250 cells/ mm^3 were significantly less likely to have patent parasitemia. Although the prevalence of malaria parasitemia was higher during the rainy season than the dry season (34/153; 22.2% vs 40/234; 17.1%), the difference was not statistically significant. In the same manner, the geometric mean parasite density though higher (640/ μl) during the high transmission rainy season than the dry season (406/ μl) was not statistically significant.

Prevalence of malaria parasite by Paracheck-Pf
Paracheck-Pf malaria RDT detected malaria in 19.3% (74/383) of the enrollees. Four patient samples (1%) yielded indeterminate results on testing with Paracheck-Pf. Microscopy failed to detect malaria parasites in all four samples that gave indeterminate

Table 3 Correlation between microscopy and selected clinical characteristics of study participants

Characteristics	Microscopy positive	P-value
Malaria treatment within two weeks		
Yes	19/124 (15.3%)	0.264
No	52/256 (20.3%)	
Temperature ($^{\circ}\text{C}$)		
Mean (\pm SD)	37.75 \pm 0.91	0.134
High temperature ($\geq 37^{\circ}\text{C}$)		
Yes	44/191 (23%)	0.69
No	29/187 (15.5%)	
Packed cell volume (%)		
Mean (\pm SD)	31.27 \pm 5.7	0.914
Anemia (PCV $<$ 33%)		
Yes	39/197 (19.8%)	0.897
No	34/177 (19.2%)	
CD4+T-cell count		
≤ 250 cells/ mm^3 (149)	35 (23.5%)	0.043
> 250 cells/ mm^3 (233)	37 (15.9%)	
Antiretroviral treatment		
Yes (267)	44 (16.5%)	0.018
No (87)	21(24.1%)	
Cotrimoxazole treatment		
Yes (174)	26 (14.9%)	0.067
No (180)	39 (21.7%)	

results. Further details of comparison of microscopy and Paracheck-Pf test results are shown in Table 4. It is noteworthy that none of the 14 enrollees who had a history of arthritis recorded false positive results on RDT testing. There was concordance between microscopy and RDT result in 317/387 cases (81.9%). There were 33 cases of false positive results and 11 (33.3%) of these had a history of recent antimalarial drug treatment within two weeks of enrollment. However, there was no correlation between a positive history of recent malaria treatment and results of RDT. Another 33 (8.5%) patients recorded false negative RDT results. Parasite density was less than 200/ μl in 84.8% (28/33) of patients with false negative RDT results while parasite density was less than 500/ μl in all but one [97% (32/33)] of the patients who tested false negative to RDT. One of the 33 patients with a false negative result had a parasite density of 20 400/ μl . The overall sensitivity of Paracheck-Pf was found to be 55.4% at all parasite densities. This rose to 90.9% and 97.6% at parasite densities $\geq 200/\mu\text{l}$ and $\geq 500/\mu\text{l}$, respectively. The specificity of Paracheck-Pf in this study was of 90.3% at parasite densities $\geq 200/\mu\text{l}$ and $\geq 500/\mu\text{l}$.

Discussion

During this study we evaluated the prevalence of malaria parasitemia among HIV seropositive adult patients suspected of having malaria at the PEPFAR clinic in Ibadan, Nigeria by microscopy and Paracheck-Pf RDT. The prevalence of malaria parasitemia was only 19.1% by expert microscopy with a geometric mean parasite density of 501/ μl . This is similar to the overall prevalence of 18.9% malaria parasitemia reported by Onyenekwe *et al.*²⁹ in a survey of asymptomatic HIV +ve persons in south-eastern Nigeria, an area of similar intensity of malaria transmission. The prevalence of malaria parasitemia among the study population is also similar to the 20.2% prevalence, which was obtained among 391 sero-negative blood donors in the same study hospital a few years earlier.³⁰

The range of parasite densities was very wide ranging from 39/ μl to 749 206/ μl with over half of the patients recording parasite densities $\leq 200/\mu\text{l}$ while almost two-thirds had parasite density less than 500/ μl . The large proportion of patients with parasite density less than 200 asexual parasites/ μl is reminiscent of the findings of an earlier study, which evaluated malaria parasitemia among healthy blood donors in the same hospital about three years before the current study in which about 76% of the parasitemic study participants recorded parasite densities $\leq 200/\mu\text{l}$.³⁰ Earlier workers have also reported low prevalence of malaria parasitemia among HIV positive persons receiving HAART and/or co-trimoxazole therapy.³¹⁻³³ Reports

of antimalarial properties of some antiretroviral drugs especially the protease inhibitors^{34,35} probably contribute to the low prevalence of malaria parasite in HIV +ve persons on HAART. French *et al.*⁴ had reported increased rates of malaria infection with deteriorating immune status. It is only reasonable to expect that the level of immune-competence will improve among patients on HAART as the CD4 count rises. This might translate to a partial if not complete restoration of the patients' earlier malaria semi-immune status.³⁶ About 70% (270/387; 69.8%) and 45.7% (177/387) of our study participants were receiving antiretroviral and co-trimoxazole, respectively.

The relatively low prevalence of malaria parasitemia in this group of HIV +ve patients who would have been diagnosed presumptively as having malaria and treated with an ACT further underscores the need for parasite based diagnosis of malaria. Given the low density of parasitemia in the majority of the patients, it is unlikely that the symptoms of over half of those who were parasitemic were due to malaria. Nakanjako *et al.*³² working in Uganda, an area of low to moderate malaria endemicity reported similar low counts in their study during which they followed up asymptomatic malaria among PLWHIV on HAART. None of the patients in the series reported by Nakanjako *et al.*³² with patent parasitemia reported signs and symptoms of malaria infection during the six months of follow up. Mills *et al.*³⁷ had also reported a markedly low incidence of malaria among HIV-positive adults who were receiving antiretroviral therapy, cotrimoxazole, and slept under ITN in rural Rakai, Uganda an area described as meso-endemic for malaria.

Performance of Paracheck-Pf

Paracheck-Pf RDT proved to be accurate and reliable for detecting malaria parasite densities of $\geq 200/\mu\text{l}$ in the population of HIV positive patients in this study. The finding that the sensitivity and specificity of Paracheck-Pf RDT increased with increasing parasite density is consistent with previous reports.³⁸⁻⁴¹ The recorded sensitivities of 90.9% and 97.6% at asexual parasite densities of $\geq 200/\mu\text{l}$ and $\geq 500/\mu\text{l}$, respectively, means that the test will give less than nine false negative results for 100 cases of *P. falciparum* at a parasite density of 200/ μl and

above while it will fail to detect only 2.5% at a parasite density $>500/\mu\text{l}$ microscopically confirmed. A further evidence for the good performance of Paracheck-Pf is the associated high negative predictive values of 98.7% and 99.7% at the two cut off parasite densities of 200/ μl and 500/ μl , respectively. While the high sensitivities allow the clinician to be confident of not missing practically any significant malaria cases, the high negative predictive values also allow the physician to confidently diagnose RDT-negative persons as non-malaria patients. This way, other causes of fever can be searched for and appropriate treatment given early. The high sensitivity and specificity obtained in this study is consistent with reports by previous workers.^{17,19,42,43}

It is, however, a matter of concern that one of the patients with a false negative RDT result had a high parasite density of 20 400/ μl . Previous workers have reported similar findings.^{17,39,44,45} Discordance between microscopy and RDT results at such high parasite densities has been attributed to a 'prozone effect'.⁴⁶ The prozone effect which is also known as high dose-hook phenomenon is defined as false negative or false low results in immunological reactions due to an excess of either antigens or antibodies. False negative results though generally associated with low parasite densities could be due to a number of other reasons. One of these reasons is that the *Plasmodium falciparum* (*Pf*) HRP-II protein has been reported to exhibit a high level of polymorphism.⁴⁷⁻⁴⁹ Polymorphism in the genes encoding the HRP-II protein is an important factor that may affect the performance of RDT based on antigen detection. The presence of non-falciparum infection will also give a false negative result as only *P. falciparum* releases HRP-II. Non-falciparum malaria may therefore be misdiagnosed as malaria negative. Hence, in geographical regions where there is the possibility of having other species of *Plasmodia* causing malaria, Paracheck-Pf would give false negative results for malaria. In such locations, an RDT with a pan-malaria antibody will be a better option. Microscopy will also be useful in complementing the HRP-II only RDT test. The proportion that may be due to non-falciparum infections in our environment will however be quite low since over

Table 4 Comparison of Paracheck-Pf malaria rapid diagnostic test versus thick smear microscopy for detection of *Plasmodium falciparum* among adult HIV +ve patients with presumptive diagnosis of malaria

Paracheck-Pf result	Malaria microscopy results		
	Positive thick smear	Negative thick smear	Total
Positive	41 (10.6%)	33 (8.5%)	74 (19.1%)
Negative	33 (8.5%)	276 (71.3%)	309 (79.8%)
Indeterminate	0 (0%)	4 (1%)	4 (1%)
Total	74 (19.1%)	313 (80.9%)	387 (100%)

95% of malaria infections in Nigeria are due to *P. falciparum*.²³ The limitation of the light microscope in detecting malaria parasite is also noteworthy and may be responsible for false negative RDT results even in expert hands.^{19,20,50} Minja *et al.*²⁰ and Batwala *et al.*⁵⁰ reported that the HRP-2 RDTs detected some cases of sub-microscopic parasitemia which were confirmed by PCR, a more sensitive method for detection of malaria parasite.

The occurrence of false positive RDT results during this study was high (33/74; 44.6%). This is consistent with findings in areas of high malaria transmission and could be due to a number of reasons, which include presence of sub-patent malaria infection, recently treated malaria infection, and rheumatoid factor positivity.^{17,39,44} In high transmission areas such as we have in south-western Nigeria, the prevalence of sub-patent malaria is high.⁵¹ False positive results may thus occur because of the limitation in the sensitivity of malaria microscopy. Some of our study participants who showed a false positive result to RDT might have had a recent malaria infection, which had been successfully treated. Delayed clearance of HRP-II following acute malaria infections is well documented.^{42,52,53} Histidine-rich protein-II based tests have been shown to remain positive for up to one month (mean time being about two weeks) after parasite clearance. Histidine-rich protein-II clearance time is a function of many factors that are not well understood. However, it has been linked to the patient's parasite density. The higher the parasitemia, the longer it takes the body to eliminate HRP-II, thus HRP-II antigenemia may persist for weeks after successful antimalarial therapy has eradicated the asexual blood stage parasites.^{38,53} It is of note that almost a third of the enrollees had a history of antimalarial drug use within two weeks of enrollment. The intensity of transmission during seasonal changes has been documented to affect the level of sensitivity and specificity in some large studies.^{17,39} Other comorbidities such as rheumatoid factor heterophilic antibodies can also result in false-positive results. Although we did not test for rheumatoid factor during this study, it is, however, noteworthy that none of the 14 patients that had a history of arthritis recorded a false positive malaria RDT result.

In conclusion, the results from this study show that the performance of Paracheck-Pf was comparable to microscopy in the diagnosis of malaria in HIV +ve patients at parasite density $\geq 200/\mu\text{l}$. This is similar to the findings of Mills *et al.*,³⁷ who evaluated the utility of Binax Now malaria RDT (an HRP-II plus pan malaria aldolase-based RDT) in Uganda. Paracheck-Pf and other malaria RDTs offer the opportunity to extend the benefits of parasite-based diagnosis beyond rural or primary health care level to a busy

PEPFAR clinic in a tertiary healthcare facility by allowing prompt decision making in the confirmation or elimination of a diagnosis of malaria. Repeat RDT testing in symptomatic patients with low counts who test negative will become positive as parasite density rises even if they were missed initially as sensitivity and specificity of HRP-II-based malaria RDT increases at higher parasite density. In this regard it is important that patients with negative test results return to the health facility for repeat testing if their symptoms have not resolved within two days of the initial consultation. The emergence and widespread dissemination of drug resistant plasmodium infection which necessitated the switch from the cheap and easily available chloroquine to the more expensive ACT¹⁵ has made RDTs more cost-effective than a decade ago.⁵⁴ In addition, RDTs are now very widely used requiring mass production with its attendant benefit of cost reduction contributing to the cost-effectiveness.

Conflict of Interest

The authors declare no conflict of interest.

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Rational case management of malaria with a rapid diagnostic test, Paracheck Pf[®], in antenatal health care in Bangui, Central African Republic

Alexandre Manirakiza^{1,2*}, Eugène Serdouma^{3,4}, Luc Salva Heredeibona⁵, Djibrine Djalle¹, Nestor Madji⁶, Methode Moyen⁶, Georges Soula², Alain Le Faou⁷ and Jean Delmont²

Abstract

Background: Both treatment and prevention strategies are recommended by the World Health Organization for the control of malaria during pregnancy in tropical areas. The aim of this study was to assess use of a rapid diagnostic test for prompt management of malaria in pregnancy in Bangui, Central African Republic.

Methods: A cohort of 76 pregnant women was screened systematically for malaria with Paracheck_{Pf}[®] at each antenatal visit. The usefulness of the method was analysed by comparing the number of malaria episodes requiring treatment in the cohort with the number of prescriptions received by another group of pregnant women followed-up in routine antenatal care.

Results: In the cohort group, the proportion of positive Paracheck_{Pf}[®] episodes during antenatal clinics visits was 13.8%, while episodes of antimalarial prescriptions in the group which was followed-up routinely by antenatal personnel was estimated at 26.3%. Hence, the relative risk of the cohort for being prescribed an antimalarial drug was 0.53. Therefore, the attributable fraction of presumptive treatment avoided by systematic screening with Paracheck_{Pf}[®] was 47%.

Conclusions: Use of a rapid diagnostic test is useful, affordable and easy for adequate treatment of malaria in pregnant women. More powerful studies of the usefulness of introducing the test into antenatal care are needed in all health centres in the country and in other tropical areas.

Keywords: Rapid diagnostic test, Malaria, Pregnancy

Background

Malaria is a major public health problem, especially during pregnancy. In 2008, malaria affected more than 2 billion people, nearly 40% of the world population. More than 50 million women live in malaria-endemic areas, of whom 20% become pregnant each year, and half of whom develop complications of pregnancy due to malaria [1,2]. Placental inter-villous sequestration of malaria parasites during pregnancy can cause growth retardation by reducing nutrient intake and causing hypoxia [3,4] and thus low birth weight [3-5]. Placental infection with *Plasmodium* is associated

with a 50% risk for low birth weight in areas where malaria is endemic [6]. Clinical malaria increases the risks for abortion, premature delivery and maternal death [7-9].

Sulfadoxine-pyrimethamine (SP) is a promising means of preventing the adverse effects of malaria, as first suggested by studies conducted in East Africa [10]. Hence, the World Health Organization (WHO) has recommended SP as a component of one of three packages to control malaria in pregnant women in Africa [11]: four antenatal visits, during which two doses of intermittent preventive (or presumptive) treatment with SP (IPTsp) spaced by at least 4 weeks are given by directly observed treatment; use of insecticide-treated nets (ITNs) to reduce the number of infective bites; and immediate, adequate treatment of clinical malaria. IPTsp involves administration of curative doses to asymptomatic women

* Correspondence: amanirak@yahoo.fr

¹Institut Pasteur de Bangui, Bangui PO Box 923 Central African Republic

²Centre de Formation et de Recherche en Médecine et Santé Tropicales, Faculté de Médecine Nord, 13015, Marseille, France

Full list of author information is available at the end of the article

(two doses for women with negative HIV status and three doses for those infected with HIV). Because SP can be embryotoxic, it should be administered only after the 16th week (second trimester) of pregnancy [12,13]. The efficacy of IPTsp plus ITNs in reducing the burden of malaria during pregnancy is well proven [14-16] and cost-effective [17-20].

Lack of compliance of health care personnel with the new strategies could hamper implementation of IPTsp in particular [11,21]. Certain aspects of health care strategies determine their acceptability in the field [22]. Thus, the long-standing practice of prescribing antimalarial drugs only on the basis of a clinical presumption of malaria could result in under-administration of IPTsp in antenatal health care services.

Bardaji et al. in Mozambique [23] found that 77.4% of women attending antenatal clinics had clinical signs or symptoms suggesting malaria, and 92.9% of these were prescribed antimalarial drugs without laboratory confirmation of *Plasmodium* infection. [24]. This practice is likely to be common in tropical areas of Africa. Our recent study in the Central African Republic showed that laboratory analysis is rare before antimalarial drugs are prescribed for pregnant women [25]. Although widespread presumptive prescription and use of antimalarial agents could indisputably lead to prompt clearance of existing peripheral and placental *Plasmodium* infection and decrease the risk for low birth weight [26], unnecessarily wide use of antimalarial drugs is avoidable if preventive methods (IPTsp and LLINs) are used. The increasing resistance of *P. falciparum* to SP [27,28] jeopardizes the ability of this drug combination to eliminate the parasite completely, especially when it is sequestered in the placenta, hence, the importance of systematic screening for malaria during pregnancy [29], and use of rapid diagnostic tests (RDTs) is a possible strategy [30,31].

In public health care practice in the Central African Republic, malaria is diagnosed by analysing thick and thin blood smears; RDTs have not yet been introduced. The aim of this study was to assess the efficacy of systematic screening for malaria with RDTs during antenatal health care in reducing over-prescription of antimalarial drugs to pregnant women. The number of episodes of malaria confirmed by a RDT in a prospective cohort of pregnant women receiving routine antenatal care was compared with the number of antimalarial prescriptions in a control group of pregnant women at the same clinics.

Method

Study setting and design

This study was conducted in the two main public maternity clinics of Bangui, Central African Republic, the Cas-tors Health Centre and the Amitié Hospital, between October 2009 and October 2011. In Bangui, the climate is

tropical, with rainfall peaks from April to November and temperatures ranging from 19 °C to 33 °C. The main malaria parasite is *Plasmodium falciparum*, and malaria transmission is endemic, with peaks during the rainy season, although no data are available on the intensity of malaria transmission. Malaria accounts for more than 40% of morbidity in the country (CAR Ministry of Health, unpublished data).

The objective of the National Malaria Programme is to reduce morbidity and mortality related to malaria in the general population, especially among children under 5 years and pregnant women, to reach a coverage rate of at least 80% with each WHO package [11]. This study consisted of strict application of the three components of the WHO package to a cohort of women during their pregnancy. Longitudinal data on *Plasmodium* infection were compared with data collected from a matched control group followed-up routinely by the antenatal services staff.

Ethical approval

Because there is no ethical committee in the country, this project was reviewed and approved by an *ad hoc* scientific committee of the University of Bangui in charge of validating scientific study protocols. The scientific committee of the 'Ecole Doctorale des Sciences de la Vie et de la Santé de l'Université de la Méditerranée, Aix-Marseille' also approved the study protocol and its amendments.

Sample size

We used 25% as a proxy for the number of malaria episodes during pregnancy [29], and 50% as a proxy for the number of women usually prescribed antimalarial treatment [25]. The estimated sample size with 80% power at the 5% significance level was therefore 60 in each group.

Procedures

Antenatal services staff were informed about the study, and we collected data in the context of usual non-malaria antenatal health care, including enrolment. During each working day, a maximum of two pregnant women from among those presenting at the clinics were randomly included in the cohort. The women were of all parities with a gestational age less than 28 weeks, from whom written informed consent was obtained. Women who were temporary residents of Bangui, had had a prior dose of IPTsp, gave a history of sensitivity to SP, quinine or an artemisinin derivative, had an illness requiring hospital admission or declined to join the study were excluded.

At enrolment, all women were given an ITN (supplied by the National Malaria Control Programme), and a finger-prick blood sample was obtained for preparation of slides and for diagnosis of *Plasmodium* infection with the Paracheck_{Pf}[®] RDT (Orchid Biomedical Systems, India).

We recorded sociodemographic data (age, address, occupational status and educational level) and also gestational age, gravidity, parity and HIV serological status.

During follow-up, an IPTsp dose (1500 mg sulfadoxine and 75 mg pyrimethamine) was administered after 16 weeks of gestation and a second dose at least 1 month later. Smear slide analysis and the RDT were performed systematically during each scheduled antenatal visit or at any unscheduled visit for women who reported malaria-like symptoms. Women with any symptom or clinical sign suggesting malaria [23] and with a positive result in the RDT and/or on a blood smear were given quinine (24 mg/kg of body mass) for 7 days. Women who reported sensitivity to quinine were given artemether 300 mg (20 mg) and lumefantrine (120 mg) if the gestational age exceeded 16 weeks. Women with a positive result in the RDT or on a thick smear but with no malaria symptoms were given one IPTsp dose; a smear was made 8 days later to verify any persistent parasitaemia or at any time earlier if a woman presented with symptomatic malaria, when quinine or artemether-lumefantrine was administered. All women were prescribed daily ferrous (400 mg) and folic (5 mg) supplements. All the antimalarial drugs were supplied by the 'Unité de Cession du Médicament', a public wholesaler that imports generic drugs.

Each woman in our cohort was matched on gravidity (1 or ≥ 2) and age (± 5 years) to another women delivering at the same maternity clinic within 1 week and from whom written informed consent was also obtained. Other criteria for enrolment in the control group were: known last date of menstruation (or gestational age at delivery), at least one antenatal visit, known HIV serological status and sleeping under an ITN. The following data were collected: socio-demographic information (age, address, occupational status and educational level), gestational age, gravidity, parity, HIV serological status, number of antenatal visits and number of malaria treatment episodes (with or without laboratory confirmation) during pregnancy.

At each antenatal visit, the RDT and blood smears were performed for each woman in the cohort. At delivery, these tests were performed on both maternal peripheral blood and placental blood. The placental blood was collected from the maternal paracentric side of the placenta after incision, and thick blood films were prepared from a droplet collected by aspiration through a 21-gauge needle attached to a 5-ml syringe [32,33]. Newborns were weighed on a mechanical infant body scale.

Laboratory analysis

The maternal and placental thick smears were air-dried, stained with 4% Giemsa and analysed under a light microscope ($\times 100$ oil immersion) to detect asexual forms of *P. falciparum*. The parasite density in maternal peripheral blood was determined from the number of parasites per

200 leukocytes on the assumption of an average leukocyte count of 8000/ μ l of blood. For maternal blood films, a result was considered negative if no parasites were detected per 200 leukocytes; for placental blood films, a result was considered negative if no parasites were detected per 100 microscope fields. All the slides were read independently by two microscopists. In case of a discrepancy, a third reading was done.

The RDT was performed according to the manufacturer's guidelines and stored at room temperature (26–32 °C). Briefly, blood samples from the finger-prick and placenta were blotted into the sample well of the test device with the loop provided in the kit. Then, six drops (almost 300 μ l) of clearing buffer were dispersed into the indicated well. The result was read after exactly 15 min. It was considered negative if a pink band appeared only in the control window and positive if, in addition to the control band, a distinct pink band also appeared in the test window. If no band appeared, the test was considered invalid and was repeated with a new device.

Data analysis

Data were double-entered into EpiInfo software version 3.5.3, and the database was checked and data entry errors corrected with the EpiInfo software 'data compare' utility for finding differences between two tables. Statistical analysis was conducted with Stata 11.2 and MedCalc v11.6.1.

Categorical variables were compared by either the χ^2 test or Fisher exact test, and quantitative variables were compared by the Student *t* test (Mann–Whitney *U* test). The level of significance (*P*) was fixed at 0.05 for all statistical tests.

We used the attributable fraction calculation procedure [34] to estimate avoidance of antimalarial drug prescription for pregnant women systematically screened for malaria. Thus, the risk for exposure to antimalarial drugs was calculated for each group, and then we calculated the relative risk (RR) of the women in the cohort for being treated in relation to the control group, and used the 1–RR formula to calculate the preventive fraction.

Results

Participant flow

During the study period, 874 pregnant women presented at their first antenatal visit. At baseline, 412 women fulfilled the inclusion criteria. A cohort sample of 83 pregnant women was randomly recruited from among women who fulfilled the inclusion criteria. The remaining 329 women were followed-up routinely by the antenatal services staff, providing an eligible population from which the control group was recruited at delivery. In the cohort group, 76 women (91.6%) were followed-up until delivery. At this end-point, this cohort group was matched to the control group (Figure 1). Overall, the total number of

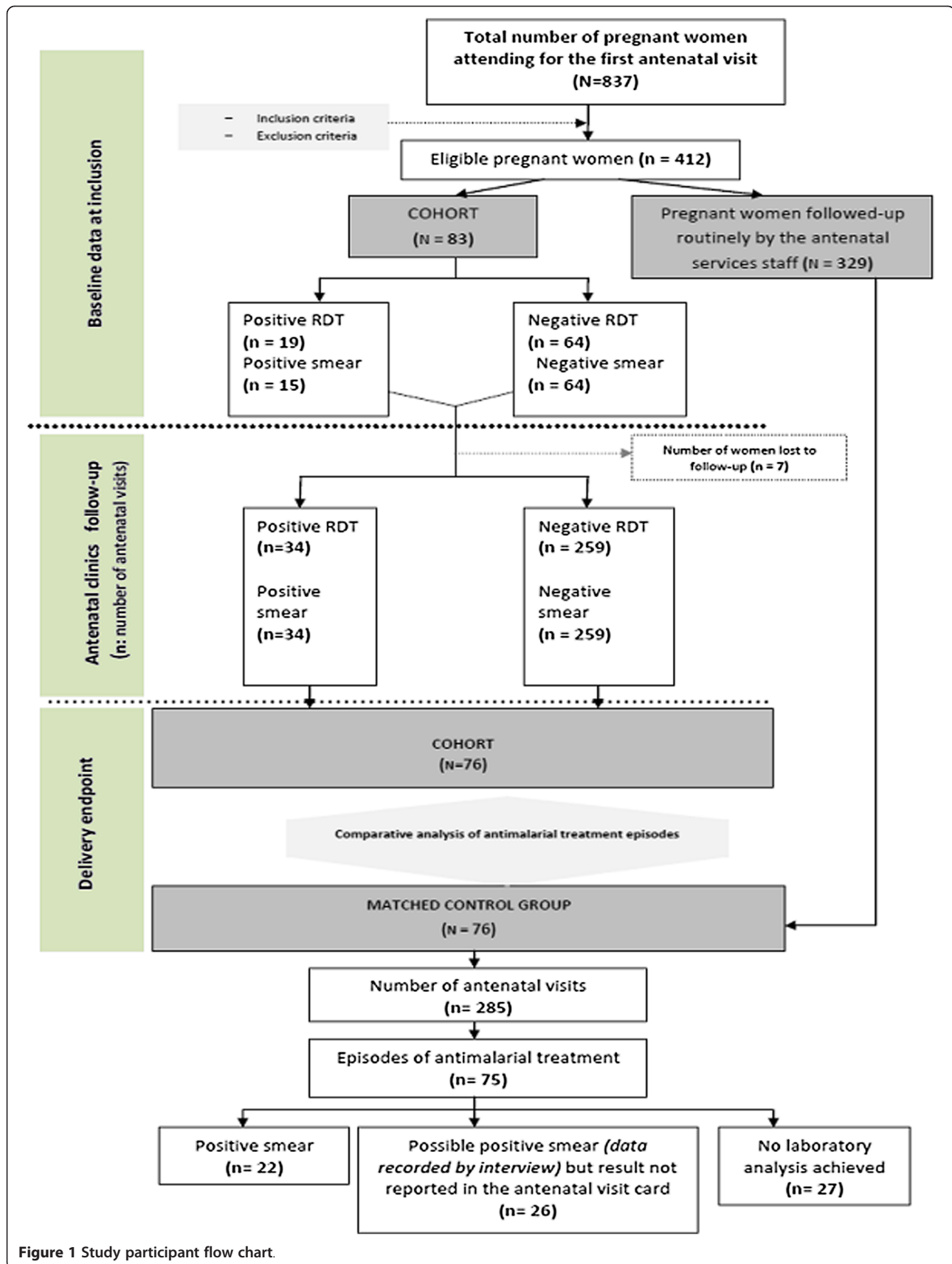


Figure 1 Study participant flow chart.

antenatal visits was 369 in the cohort group and 285 in the control group.

Characteristics of the study population

The average age of the women was 26 years (SD = 5 years), and the mean gravidity was 2 (range, 1–6), with no statistically significant differences between the groups. The serological prevalence of HIV infection was 7.6% (Table 1). The distribution of visits by length of gestation (in months) is shown in Figure 2. The IPTsp coverage rate with at least two doses was 93.4% in the cohort and 65.8% (50/76) in the control group. A statistically significant difference in the mean weight of birth of the infant was found between the two groups ($P = 0.007$).

Results of diagnostic tests for malaria

In the cohort, 40 pregnant women (52.6%) presented clinical signs at baseline suggesting malaria. Of these women, 42.5% (17/40) had a positive RDT for malaria; none of the asymptomatic pregnant women had a positive result for malaria. During follow-up, 293 antenatal visits were made by the cohort group, and symptoms suggesting malaria were noted in 35.5% (104/293); however, positive RDT were found for only 29.8% (31/104). Overall, positive results for *P. falciparum* infection were found by the RDT at 13.8% of antenatal visits (51/369) and by thick blood smear analysis at 11.6% (43/369) of visits. Concordance was found for positive results in the RDT and in thick blood smears but not for negative results, as eight results were negative in the thick blood smear but positive with the RDT (15.7% discordance). Figure 1 shows a flow chart of these diagnostic tests.

Women positive results in the RDT were treated with either artemether-lumefantrine (45.1%, 23/51) or quinine

(54.9%, 28/51). During follow-up, three asymptomatic malaria episodes were seen, with positive results in both the RDT and thick blood smear analysis. Each of these women received one dose of SP, and thick blood smears analysed 8 days later and at the following scheduled visit was negative.

In the control group, 75 prescriptions for a possible malaria episode were delivered by the antenatal services staff. Positive thick blood smears were documented on 35.0% of antenatal visit cards. These cards showed that 29.3% (22/75) prescriptions of antimalarial drugs were achieved after a documented positive laboratory result for malaria before treatment, while 36.0% (27/75) antimalarial drugs were achieved without laboratory analysis. For remaining prescriptions, laboratory analysis was not recorded in antenatal visit cards. However, interviewed women were reported that smear analysis were positive.

At delivery, the results of the two tests were concordant for both the maternal peripheral blood smear and the placental blood samples in the two groups. Four placental blood samples from the cohort and only two from the control group were positive in the RDT. In the control group, five placental blood samples and two blood slides were positive with the RDT (Figure 3).

During this study, none of the tests was invalid.

Effect of RDT results on malaria treatment

Only 13.8% (51/369) of the pregnant women in our cohort needed antimalarial treatment after a positive RDT, while 26.3% (75/285) of the control group received such prescriptions. Antimalarial treatment was significantly more frequent in the control group than in the cohort ($P = 0.0001$). The relative risk of the cohort for being prescribed an antimalarial drug was 0.53; therefore, 47% of prescriptions were

Table 1 Characteristics of the study population

Characteristic	Cohort (N = 76)	Control group (N = 76)	P value
Mean age (years) (SD)	25.9 (5.4)	26.7 (3.8)	NS
Number of pregnancies			
0–1	32.4 (23)	29.6 (21)	NS
2	25.4 (18)	26.8 (19)	NS
≥ 3	49.3 (35)	50.7 (36)	NS
Educational level			
None	18.3(13)	14.1 (10)	NS
Primary school	33.8 (24)	38.0 (27)	NS
Secondary school	54.9 (39)	54.9 (39)	NS
HIV serological status	7.9 (6)	5.3 (4)	NS
Birth weight, mean g (SD)	3161 (351)	3297 (261)	0.007
Proportion of infants weighing < 2500 g	5.3	0.0	-

SD, standard deviation

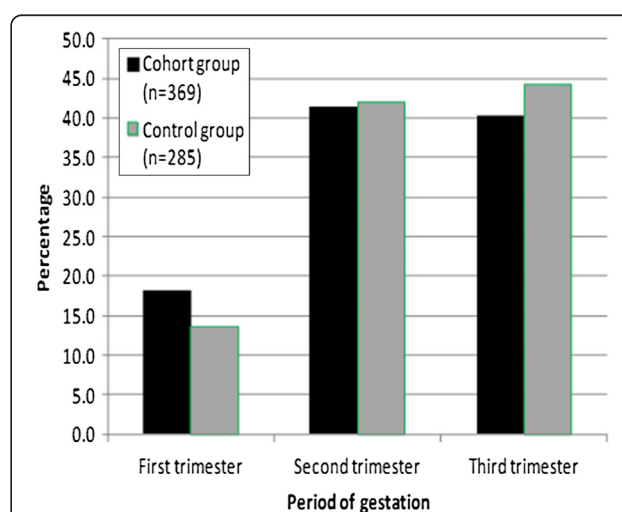
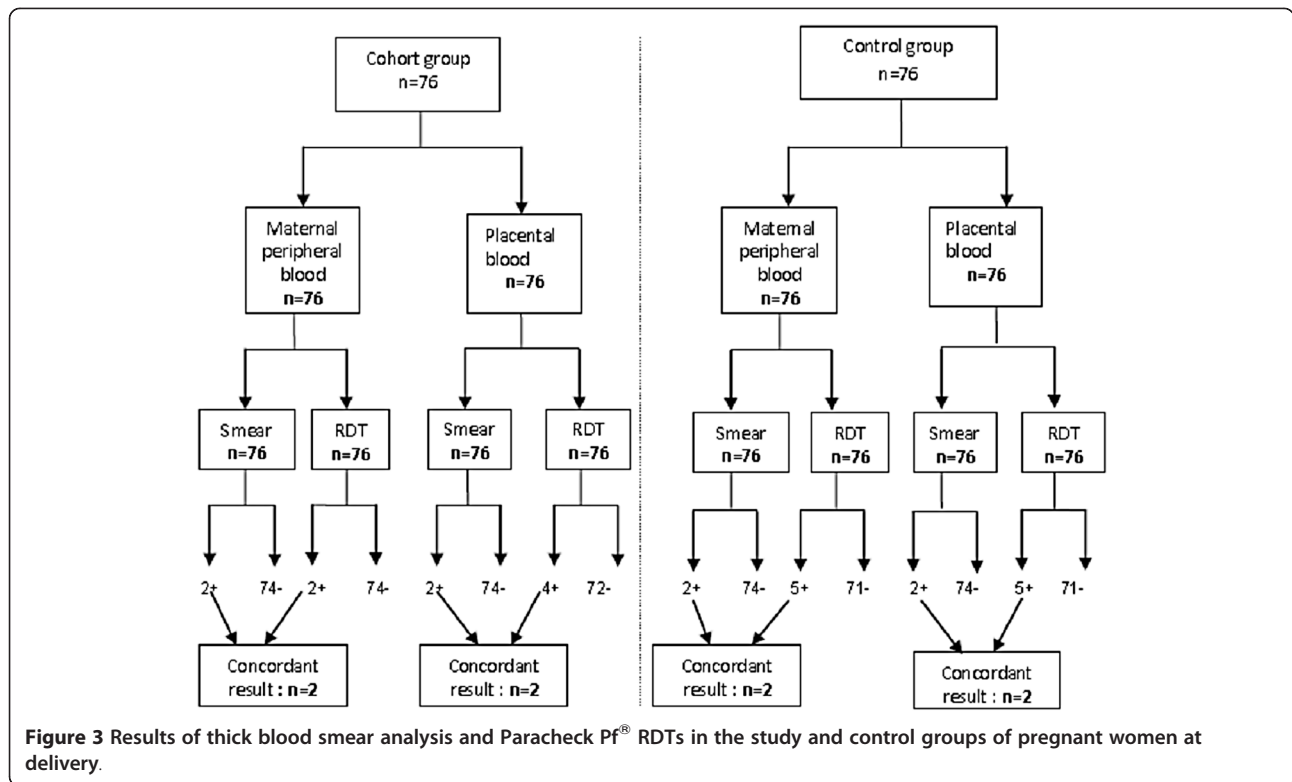


Figure 2 Numbers of antenatal visits according to gestational age in the cohort and control groups of pregnant women.



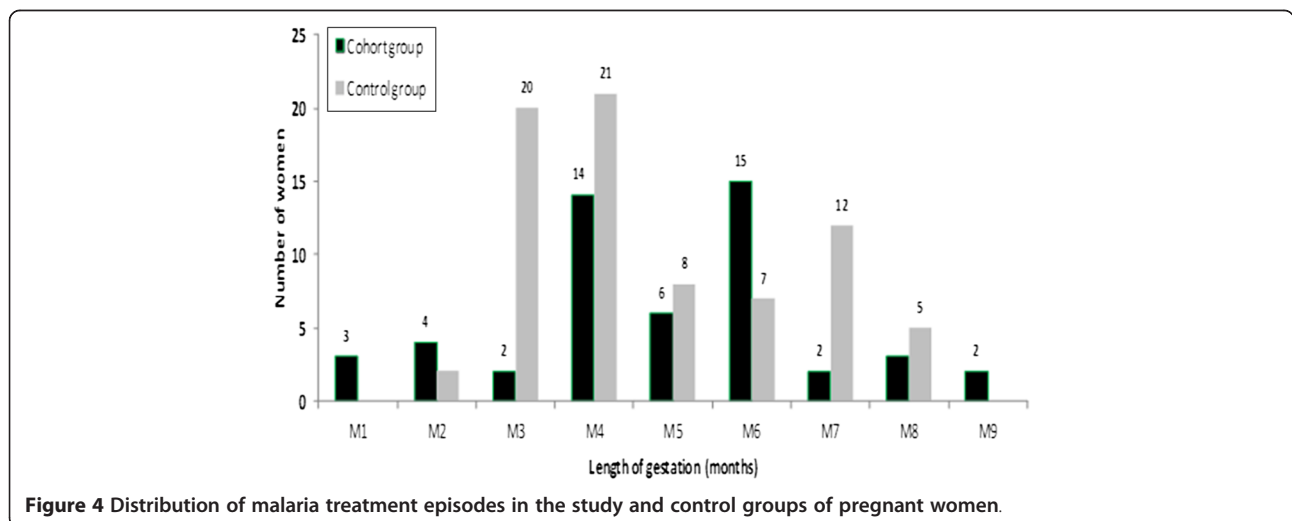
avoided with use of the RDT. The distribution of malaria treatment according to gestational age was, however, similar in the two groups (*U*-test *P* values > 0.05) (Figure 4).

Discussion

Our findings highlight the value of using an RDT in managing malaria during pregnancy. The usefulness of RDTs in the diagnosis of malaria in patients presenting with symptoms or clinical signs is well established [35,36], although there has been concern that the number of malaria

cases might be overestimated [37,38]. The performance of the ParacheckPf[®] RDT in the diagnosis of malaria in pregnancy was recently appraised [39], and the use of RDTs in reducing the cost of treatment of malaria as compared with presumptive treatment has been demonstrated in children in Cameroon [40]. However, heat stability, vital to maintaining the sensitivity of this test in the field, is a concern [41,42], although there were no invalid tests.

The ParacheckPf[®] RDT was more sensitive than microscopic analysis of thick blood smears, as found in other



studies [38-40]. The priority is to determine whether an RDT can be positive when microscopy is negative [43]. The location of parasites in the deep blood circulation, particularly in the placenta, with release of antigens into the systemic circulation would explain a negative result in thick blood slides when an RDT is positive.

We observed a significant reduction in the number of antimalarial drug prescriptions in pregnant women when the ParacheckPf[®] RDT was used at each antenatal visit. This strategy therefore reduces unnecessary expenditure on drugs and also reduces unwarranted exposure of pregnant women to those drugs. Moreover, appropriate early treatment of malaria during pregnancy helps to eliminate parasites from both maternal blood and the placenta.

It has been estimated that malaria parasites are present in the placenta in only 20% of cases [44,45]. In our study, this proportion was 15.7%. As parasites sequestered in the placenta release antigens into the peripheral blood, they can be detected by RDT at any time of pregnancy.

One potential limitation of our study is the small sample, which precluded further analysis of birth weights; however, a study of three cohorts of a total of 3333 women conducted in Ghana [29] led to the same conclusion regarding the usefulness of screening with an RDT and treatment of positive cases. A second potential limitation was possible bias due to the "Hawthorne effect", whereby people improve an aspect of their behaviour because they know they are being observed [46]. As our study was conducted in the office used by the staff of the antenatal clinics, they might have changed their attitude to improve the diagnosis of malaria by increasing their requests for laboratory analysis of thick blood smears.

Conclusion

This study shows that routine screening of pregnant women for malaria can avoid unnecessary prescription of drugs. As any use of medical products during pregnancy should be avoided, the proportion of avoidable antimalarial drug exposure that we observed in this study could be important if it were extrapolated to other primary health care services.

Ensuring clearance of malaria parasites is essential for the control of malaria during pregnancy. Thus, the ease of use and relatively high sensitivity of the ParacheckPf[®] RDT in comparison with microscopy could allow prompt, radical treatment of malaria. Its integration into primary health care services should, however, be accompanied by regular supervision of the activities of the personnel involved. More studies are needed in other areas of the Central African Republic and other countries to assess the introduction of RDTs in malaria control in pregnant women.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AM, GS and JD conceived the study. AM organized and managed the study day-to-day and contributed to writing the manuscript. DD managed the RDT kits. ES, LSH and NM participated in field data collection. All authors contributed to the preparation of the manuscript and have approved the final version.

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Author details

¹Institut Pasteur de Bangui, Bangui PO Box 923 Central African Republic. ²Centre de Formation et de Recherche en Médecine et Santé Tropicales, Faculté de Médecine Nord, 13015, Marseille, France. ³Reproductive Health, Ministry of Public Health, Population and AIDS Control, PO Box 883, Bangui, Central African Republic. ⁴Faculty of Health Sciences, University of Bangui, PO Box 1383, Bangui, Central African Republic. ⁵Ministry of Public Health, Population and AIDS Control, Castors Health Centre, Bangui, Central African Republic. ⁶Malaria Programme Division, Ministry of Public Health, Population and AIDS Control, PO Box 883, Bangui, Central African Republic. ⁷Hôpital de Brabois Adultes, CHU de Nancy, 54511, Vandoeuvre-lès-Nancy Cedex, France.

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Short Report: Performance of a Histidine-Rich Protein 2 Rapid Diagnostic Test, Paracheck Pf[®], for Detection of Malaria Infections in Ugandan Pregnant Women

Mehul Dhorda,* Patrice Piola, Dan Nyehangane, Benon Tumwebaze, Aisha Nalusaji, Carolyn Nabasumba, Eleanor Turyakira, Rose McGready, Elizabeth Ashley, Philippe J. Guerin, and Georges Snounou
Epicentre, Mbarara, Uganda; Unité Mixte de Recherche 945, Institut National de la Santé et de la Recherche Médicale, Paris, France; Université Pierre et Marie Curie, Faculté de Médecine Pitié-Salpêtrière, Paris, France; Epicentre, Paris, France; Centre for Clinical Vaccinology and Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom; Mbarara University of Science and Technology, Mbarara, Uganda; Shoklo Malaria Research Unit, Mae Sot, Tak, Thailand

Abstract. Improved laboratory diagnosis is critical to reduce the burden of malaria in pregnancy. Peripheral blood smears appear less sensitive than *Plasmodium falciparum* histidine-rich protein 2–based rapid diagnostic tests (RDTs) for placental malaria infections in studies conducted at delivery. In this study, 81 women in Uganda in the second or third trimester of pregnancy were followed-up until delivery. At each visit, peripheral blood was tested by blood smear, RDT, and nested species-specific polymerase chain reaction (PCR). Sensitivity and specificity of the tests was calculated with PCR, which detected 22 infections of *P. falciparum*, as the gold standard. The sensitivity and specificity of blood smears were 36.4% (95% confidence interval [CI] = 18.0–59.2%) and 99.6% (95% CI = 97.7–100%), respectively. The corresponding values for RDT were 31.8% (95% CI = 14.7–54.9%) and 100% (95% CI = 98.3–100%). The RDTs could replace blood smears for diagnosis of malaria in pregnancy by virtue of their relative ease of use. Field-based sensitive tests for malaria in pregnancy are urgently needed.

Pregnant women are highly susceptible to malaria infection and disease, which frequently leads to placental parasitemia, maternal and infant anemia, and low birth weight, all of which directly or indirectly increase the risk of maternal, fetal and infant death.^{1,2} The adverse effects of *Plasmodium falciparum* malaria in pregnancy are more marked in paucigravidae because of lack of previous exposure, and thus immunity, to parasite strains that can express receptors specific for placental sequestration.^{3,4} The latter factor is reflected in the observed reduction of incidence of microscopically detectable infections in peripheral blood and of symptomatic malaria episodes with increasing gravidity.⁵ Absence of detectable peripheral parasitemia and of symptoms can hide placental infections, which are linked to some of the more severe effects of malaria in pregnancy.⁶ Improved detection of such infections is critical for the reduction of the burden of malaria in pregnancy.⁷

Peripheral blood smears are poorly sensitive for malaria infections in pregnant women compared with the polymerase chain reaction (PCR) in high-transmission settings.^{5,8,9} Cross-sectional studies conducted at delivery in these contexts have shown that rapid diagnostic Tests (RDTs) detecting *P. falciparum* histidine-rich protein-2 (HRP2) are more sensitive than blood smears and appear to be reliable predictors of adverse outcomes of malaria in pregnancy.^{8,10,11} In the current study, women in the second or third trimester of pregnancy had frequent intermittent screening with blood smears, RDT, and species-specific nested PCR up to delivery. Standard diagnostic test indicators were determined for blood smears and RDTs with PCR as the gold standard.

The study was conducted concurrently with an ongoing randomized non-inferiority trial of Coartem[®] versus quinine for the treatment of uncomplicated malaria in pregnancy (Malaria in Pregnancy [MIP] study).¹² The protocols for these studies were approved by the Faculty Research Ethics Committee and

the Institutional Review Board of the Mbarara University of Science and Technology (Mbarara, Uganda), and the Ugandan National Council for Science and Technology (Kampala, Uganda). The MIP study protocol was also approved by the Comité de Protection des Personnes (Ile-de-France XI, Paris, France).

One hundred three pregnant women in the second or third trimester, of whom 31 also participated in the MIP study, were included in this study after documented informed consent was obtained. Women attending the Mother and Child Health Department of the Mbarara Regional Referral Hospital in Mbarara, Uganda were screened with RDTs and blood smears to detect peripheral malaria parasitemia. Slide-confirmed malaria episodes were treated with Coartem[®] or quinine. The pregnant women were followed-up, and all three tests were performed at weekly (pregnant women in the MIP study) or monthly (other pregnant women) intervals until delivery.

This study was conducted from October 2008 to June 2009. At inclusion and at every follow-up visit, blood samples were collected by fingerprick or by venipuncture if other tests were conducted for the MIP study. Blood samples were collected with EDTA as the anticoagulant. Fingerprick blood was collected in Microtainers[®] and venous blood in Vacutainers[®]. Blood smears were prepared and the RDT was performed immediately after blood collection. The remaining blood was centrifuged to harvest the erythrocyte pellet, which was stored at –80°C until PCR analysis. Giemsa-stained slides (10% v/v) were read at 1,000× magnification; 200 fields of the thick blood smear were read before declaring a slide negative. Ten percent of negative slides and all slides positive for trophozoites and/or gametocytes were read by two microscopists unaware of each other's results. A third read was performed to resolve cases of discordance (positive/negative, parasitemia difference > 50%, different species). The reported parasitemia was the average of the two closest results calculated as described.¹³

Paracheck Pf[®] (Orchid, Goa, India), an HRP2-based RDT, was procured directly from the manufacturer and performed according to the accompanying instructions. Procedures for preparation of blood samples for PCR were adapted from a

*Address correspondence to Mehul Dhorda, 11th Floor, Chamlong Harinasuta Building, 420/6 Ratchawithi Road, Ratchathewi, Bangkok 10400, Thailand. E-mail: mehul.dhorda@gmail.com

published method.¹⁴ Nested PCRs were performed initially to screen for samples containing *Plasmodium* as described.¹⁵ Species of *Plasmodium* in positive samples were determined in separate species-specific nest 2 reactions.

All data were double-entered in Epidata version 3.1 (www.epidadata.dk) and cleaned by using Stata release 10 (Stata Corp LP, College Station, TX). Because pregnant women with a positive RDT result were encouraged to join the cohort, data obtained < 28 days after a positive RDT result at inclusion were removed from the analysis to eliminate selection bias. Results obtained < 28 days after treatment with Coartem® or Quinine were also censored to remove the effect of persistent antigenemia. Further, because treatment with inefficacious drugs or drugs with some antimalarial activity can reduce parasitemia to submicroscopic levels, data obtained < 28 days from treatment with sulfadoxine-pyrimethamine and all data from pregnant women treated with cotrimoxazole prophylaxis were censored. Finally, results from PCR-confirmed pure non-*P. falciparum* or indeterminate species infections were not included in calculations. Calculations of sensitivity and specificity for blood smears and RDTs with PCR as the gold standard and comparisons of results were performed by using Epicalc version 1.02 (<http://www.brixtonhealth.com/epicalc.html>).

Results for the three tests were available from 487 pregnant woman visits. Data from 299 visits of 81 pregnant women were analyzed after censoring. Each pregnant woman had a median of 3 visits (range = 1–18 visits). There was no significant difference in percentage positivity by blood smear, RDT, or PCR in pregnant women followed-up weekly or monthly and there were no indications of variation in RDT performance. Species-specific nested PCR detected 22 *P. falciparum* infections in samples collected at these visits, of which only 8 and 7 were detected by blood smear and RDT, giving a sensitivity of 36.4% (95% confidence interval [CI] = 18.0–59.2%) and 31.8% (95% CI = 14.7–54.9%), respectively (Figure 1). The sensitivities of the two tests were not significantly different. Only one instance of a false-positive result was detected by blood smear but at a low parasitemia of 32 parasites/ μ L, giving a specificity of 99.6% (95% CI = 97.7–100%) for this test. Among 120 samples collected at visits < 28 days after antimalarial drug intake, PCR detected 20 *P. falciparum* infections, of which 9 were also detected by RDTs but only 3 by blood smears. Two false-positive results were also detected by RDTs.

The results reported are, to our knowledge, the first from a longitudinal study performed to evaluate RDTs in pregnant women before delivery. The low sensitivity of blood smears for detection of malaria in pregnant women obtained here is comparable to that in earlier reports. However, the estimated sensitivity of the RDT is similar to that of blood smears and lower than published estimates for HRP2-based tests, which were obtained from studies on samples collected only at delivery.^{8,10,11} The difference may be explained by longitudinal follow-up conducted in the current study, which enabled recording of antimalarial drug intake.

Elimination of all data obtained < 28 days from intake of drugs with antimalarial activity removed the bias which could potentially be introduced by persisting parasite antigen and/or by reduction of parasitemia to submicroscopic levels. The latter factor is especially pertinent for cotrimoxazole, which is commonly used to prevent opportunistic infections, and sulfa-

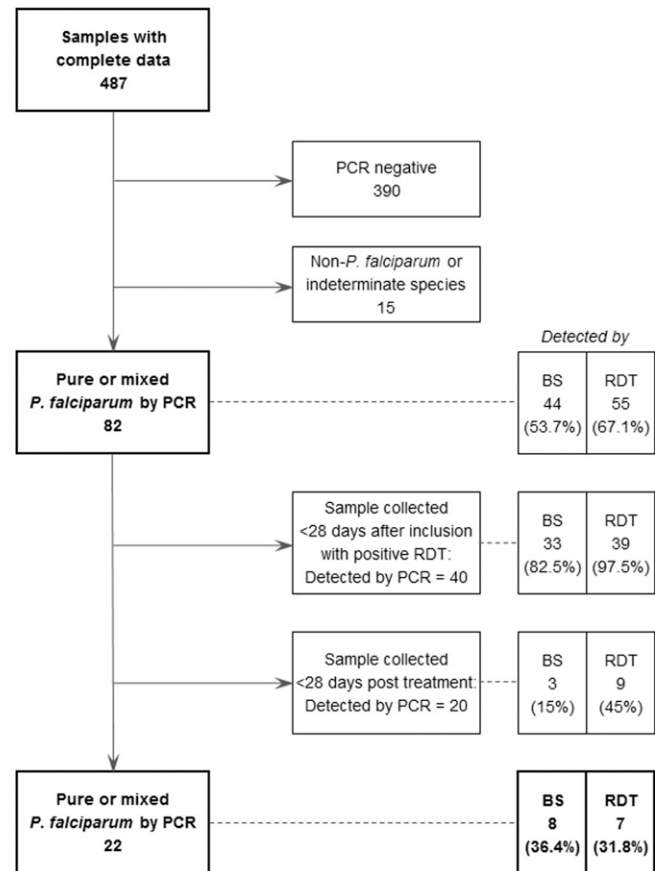


FIGURE 1. Pure or mixed *Plasmodium falciparum* infections detected by polymerase chain reaction in pregnant women, Uganda.

doxine-pyrimethamine, which is used for intermittent preventive therapy in pregnancy in Africa, which can cause malaria infections to remain at levels detectable by PCR but not by microscopy.⁵ In an RDT evaluation study in Ghana where antimalarial drug intake was also checked by an enzyme-linked immunosorbent assay in plasma, 35% of women had traces of pyrimethamine.⁸ It would be interesting to know the sensitivity of RDTs in this study if data from these women and from any others who reported antimalarial intake were censored.

Of the 9 samples collected < 28 days after antimalarial drug intake and showing positive results by RDT and PCR, 3 were collected ≤ 7 days after drug intake for a smear-positive malaria episode and likely contained persistent antigen. All three tests showed positive results for two samples. Of the remaining four samples, none of which were positive by blood smear, two were collected ≥ 14 days after drug intake for a blood smear-positive malaria episode with at least one intervening visit/sample where the RDT result was negative. The RDT showed a positive result 10 days before a microscopically detectable episode in one case and blood smears did not become positive up to delivery in one case.

In combination with the high specificity of RDTs (100% in data obtained after censoring and 98% in samples collected < 28 days after drug intake), these data appear to confirm that RDTs can detect low-level infections undetectable by microscopy in pregnant women.¹⁶ Furthermore, it enables speculation on use of RDTs for treatment monitoring in pregnant women if performed > 28 days after drug intake to reduce the possible

effect of high initial peripheral blood parasitemia on antigen persistence. If the RDT result is positive after this interval, it may indicate a continuing placental infection requiring additional treatment.

In conclusion, our results indicate that RDTs would be suitable for replacing blood smears screening and treatment of malaria in pregnancy in this malaria-endemic setting by virtue of their ease-of-use compared with microscopy. The RDTs may also have some utility in monitoring treatment. However, given the small population assessed here, these findings need to be confirmed in statistically robust studies conducted in settings of different endemicities and with other RDTs, some of which outperformed Paracheck Pf® in systematic testing.¹⁷ It would be important in such studies to take into consideration the previous drug history. Field-based diagnostic tools with improved sensitivity are urgently required for diagnosis of malaria during pregnancy.

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Authors' addresses: Mehul Dhorda, 11th Floor, Chamlong Harinasuta Building, Ratchathewi, Bangkok, Thailand, E-mail: mehul.dhorda@gmail.com. Patrice Piola and Philippe J. Guerin, Centre for Tropical Medicine, University of Oxford, Churchill Hospital, Old Road, Oxford, United Kingdom, E-mails: patrice.piola@wwarn.org and philippe.guerin@wwarn.org. Dan Nyehangane, Benon Tumwebaze, Aisha Nalusaji, Carolyn Nabasumba, and Eleanor Turyakira, Epicentre Mbarara, Mbarara, Uganda, E-mails: dan.nyehangane@epicentre.msf.org, benontumwebaze@yahoo.com, aishanalusaji@yahoo.com, carolyn.nabasumba@epicentre.msf.org, and eleanor.turyakira@epicentre.msf.org. Rose McGready, Shoklo Malaria Research Unit, Mae Sot Tak, Thailand, E-mail: Email: rose@shoklo-unit.com. Elizabeth Ashley, Epicentre, Paris, France, E-mail: e.ashley@doctors.org.uk. Georges Snounou, Unité Mixte de Recherche 945, Institut National de la Santé et de la Recherche Médicale, Paris, France and Université Pierre et Marie Curie, Faculté de Médecine Pitié-Salpêtrière, 91 Boulevard de l'Hôpital, Paris, France, E-mail: georges.snounou@upmc.fr.

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Comparison of Paracheck Pf® test with conventional light microscopy for the diagnosis of malaria in Ethiopia

Zinaye Tekeste^{1*}, Meseret Workineh¹, Beyene Petros²¹School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, Gondar University, P.O. Box 196, Gondar, Ethiopia²Department of Biology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

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ABSTRACT

Objective: To assess the accuracy of Paracheck Pf® in reference to the conventional light microscopy. **Methods:** A total of 400 patients visiting Awash, Methara and Ziway malaria centers were simultaneously screened with both light microscopy and Paracheck Pf® for the presence of *Plasmodium falciparum* (*P. falciparum*) malaria. **Results:** Of the 190 samples that were negative by light microscope, the Paracheck Pf® showed 11 false positive and 179 true negative results, and from a total of 210 samples positive by light microscope, Paracheck Pf® accurately diagnosed 200 true malaria cases. Taking the light microscopy as a standard test for malaria, the sensitivity, specificity, positive predictive value and negative predictive value of Paracheck Pf® is 95.2% [confidence interval (CI)=92.4–97.1], 94.2% (CI=91.1–96.3), 94.8% (CI=92.0–96.7) and 94.7% (CI=91.6–96.8), respectively. **Conclusions:** Paracheck Pf® showed good sensitivity and specificity for the diagnosis of *P. falciparum* malaria, and fulfill the world health organization (WHO) recommendation that requires the sensitivity of rapid diagnostic tests (RDTs) to be greater than 95%. Therefore, Paracheck Pf® can be used as an alternative to the Giemsa stain light microscopy in resource poor set ups.

1. Introduction

Malaria is caused by a parasite called *Plasmodium*, which is transmitted via the bites of infected female *Anopheles* mosquitoes. It is one of the major tropical diseases adversely affecting the health of the peoples and the economic development of many developing countries[1]. Each year, between 300–500 million malaria cases and up to 3 million deaths occur throughout the world, Africa accounting for more than 90% of the burden[2–4]. In Ethiopia, malaria remains the leading public health problem where an estimated 68% of the population lives in malarious areas and 75 % of the total land mass is regarded as malarious[5].

Employing an integrated and comprehensive approach that includes early case detection, selective vector control, epidemic management and control, environmental management and personal protection through the use of

insecticide-treated bed nets (ITBs) are the main malaria control strategies in Ethiopia[6,7]. Despite recent efforts to control the disease in the country, malaria is still the leading cause of mortality and morbidity in Ethiopia[5].

Although there are several factors that hinder effective malaria control in Ethiopia, absence of reliable method of diagnosis is the major one. In Ethiopia, malaria parasite has been diagnosed by the use of Giemsa stained microscopy. However, since parasitological diagnosis is not accessible in some rural areas of the country diagnosis of cases is accomplished through clinical diagnosis[6]. Clinical diagnosis may result in misdiagnosis (presumptive treatment of all fevers as malaria) and inappropriate use of malaria drugs[8,9]. Furthermore, poor diagnostic standards such as the lack of enough skilled microscopists and inadequate or absence of quality control systems continue to hinder effective malaria control in the country. This shows that the development of a more rapid, sensitive, easy and specific diagnostic method could substantially improve malaria control in Ethiopia.

Several investigations have been conducted to assess the accuracy of different rapid diagnostic tests (RDTs) in reference to the conventional light microscopy, and these studies have reported contradictory results. While some

*Corresponding author: Zinaye Tekeste, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, Gondar University, P.O. Box 196, Gondar, Ethiopia.

E-mail: zinzn98@yahoo.com

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studies reported a good performance of RDTs, other studies clearly indicated poor performance of RDTs^[10–13]. Therefore, the contradictory reports on the operational characteristics RDTs prompted investigation of the situation in the malaria endemic localities in Ethiopia.

2. Materials and methods

2.1. Study site

The present study was carried out in three Ethiopian malaria endemic localities (Awash, Metehara and Ziway) to assess the accuracy of Paracheck Pf® in reference to the conventional light microscopy. Malaria is seasonal in the study areas with a frequent occurrence of epidemics, often from September to December, following the heavy rainfall season. There is one malaria centre in each region where people with symptoms suggestive of malaria obtain free services for malaria diagnosis and treatment.

2.2. Study population

A total of 400 patients visiting Awash, Methara and Ziway malaria centers between November 2008 and January 2009 were included in the study. Malaria patients who had received anti-malarial treatment within 48 hours prior to confirmation of their malaria and patients co-infected with *Plasmodium falciparum* (*P. falciparum*) and other species of *Plasmodium* parasite were excluded from the study. Patients critically ill and unable to respond for the interview were also excluded from the study.

2.3. Giemsa-stained blood film

Finger-prick samples were collected and placed in grease-free clean glass slid. Each blood smear was stained with Giemsa and examined immediately under the oil immersion microscope objective by two experienced laboratory technicians. The technicians were not told about the health and other status of the study participants. In cases where the results were discordant, a third expert reader was used. The results of the third expert reader were considered the final result.

2.4. Paracheck Pf® test

Approximately 5 µL of blood sample was transferred directly from the finger or toe of the study participants into each sample well and approximately 6 drops of buffer were added. After 15 minutes the results were read as recommended by the manufacturers (Orchid Biomedical System, Verna Goa, India).

2.5. Statistical analysis

The collected data was computerized using excel program, exported and analyzed by SPSS version 16 and JavaStat two way contingency table. Sensitivities, specificities, and positive and negative predictive values were calculated using Giemsa stained microscopy as a gold standard.

2.6. Ethical clearance

Ethical clearance was obtained from the Ethical Review Committee of Department of Biology, Addis Ababa University. Written informed consent was obtained from all study participants and mothers/caretakers of children under 18 who participated in the study after explaining the purpose and objective of the study.

3. Results

During the study period, 400 febrile patients were screened for *P. falciparum* infection with both Paracheck Pf® and Giemsa stained microscopy (Table 1). Of these, 210 were found to be positive and 190 were found to be negative for *P. falciparum* malaria by light microscopy. The Paracheck Pf® detected 10 negative samples that were positive by light microscope. Giemsa stained microscopy detected 200 positive results that were also positive by Paracheck Pf® (Table 1).

Table 1

Paracheck Pf® results compared to the reference Giemsa stained light microscopy.

Test and result	Light microscopy		Total
	Positive	Negative	
Paracheck Pf® Positive	200	11	211
Negative	10	179	189
Total	210	190	400

Of the 190 samples that were negative by light microscopy, the Paracheck Pf® gave 11 false positive results, indicating 94.2% (91.1–96.3) Paracheck Pf® specificity. Furthermore, taking the Giemsa stained microscopy as a standard test for malaria, the sensitivity, positive predictive value (PPV) and negative predictive value (NPV) of Paracheck Pf® is 95.2% [confidence interval (CI)=92.4–97.1], 94.8% (CI=92.0–96.7) and 94.7% (CI= 91.6–96.8), respectively.

4. Discussion

The results of this study have shown that, the sensitivity and specificity of Paracheck Pf® was 95.2% and 94.2%, respectively. However, a relatively high Paracheck Pf® sensitivity was detected in many populations, especially those living in malaria endemic areas^[14,15]. Paracheck Pf® showed 100% sensitivity when compared to microscopy, as reported by Swarthout *et al*^[14] from amongst children aged 6–59 months in eastern Democratic Republic of Congo. Furthermore, Sharew *et al*^[15] examined 668 febrile patients who were identified in Wondo Genet, southern Ethiopia, where *P. falciparum* is endemic and found 99.4% Paracheck Pf® sensitivity. In this study, although Paracheck Pf® is found to have a relatively low sensitivity, it fulfills the WHO recommendation that requires the sensitivity of RDTs to be 95%^[16]. Therefore, having this sensitivity Paracheck Pf® can be used as an alternative to light microscopy in resource poor set ups.

In the present study, it was also shown that Paracheck Pf® failed to detect 10 cases, which were positive by microscopy. This is not in consistence with similar study in eastern Democratic Republic of Congo^[14], where all samples positive by light microscopy were also positive by Paracheck Pf®. The mechanism that RDTs cause false negative result is not

fully understood. However, a logical explanation provided on deletion or mutation of the histidine-rich protein 2 (HRP-2) gene^[17]. Some studies have clearly established the deletion or mutation of HRP-2 in patients with falciparum malaria. Also, this deletion or mutation of HRP-2 is associated with false negative results of RDTs^[17]. Therefore, deletion or mutation of HRP-2 may have been responsible for the conflicting reports on false negative results of RDTs.

In this study, it has been observed that Paracheck Pf® detected 11 cases which were negative by microscopy. Individuals living in malaria endemic areas who experience repeated malaria infections develop a degree of immunity that confers some protection from complicated malaria such as parasitemia^[18,19]. And this has been linked with the presence of samples with parasite density below the detection threshold for microscopy^[19]. However, unlike Giemsa stained light microscopy, Paracheck Pf® detects HRP-2 which could be produced in such low level of parasites^[14,19,20]. Therefore, since the present study was conducted in malaria endemic area, the false positive results obtained may be due to the presence of parasite density below the detection threshold for microscopy and detectable level of HRP-2 in some of the study participants.

In conclusion, Paracheck Pf® showed good sensitivity and specificity for the diagnosis of *P. falciparum* malaria, and fulfill the world health organization (WHO) recommendation that requires the sensitivity of rapid diagnostic tests (RDTs) to be greater than 95%. Therefore, Paracheck Pf® can be used as an alternative to the Giemsa stain light microscopy in resource poor set ups.

Conflict of interest statement

We declare that we have no conflict of interest.

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ORIGINAL ARTICLE**PARACHECK-PF[®] TEST VERSUS MICROSCOPY IN THE DIAGNOSIS OF *FALCIPARUM* MALARIA IN ARBAMINCH ZURIA WOREDA OF SOUTH ETHIOPIA****Hussein Mohammed¹, Moges Kassa¹, Amha Kebede¹, Tekola Endeshaw²****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[®] 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[®], 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).

¹Ethiopian Health and Nutrition Research Institute P. O. Box. 1242, Addis Ababa

²The Carter Center - Ethiopia

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 μl of blood, assuming an average of 8,000 WBCs/ μl of blood (11).

RDT paracheck Pf[®] (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 / μ 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/ μ l) was smaller 28.8% (15/52) than the proportion of high parasite density (20000-100000 parasite/ μ 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[®] 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 μ 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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Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex Post Office, Goa - 403202, INDIA.
Tel.: +91 832 2458546-50 Fax : +91 832 2458544 E-mail : sales@tulipgroup.com Website : www.tulipgroup.com