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Evaluation of the performance of CareStart™ Malaria Pf/Pv Combo rapid diagnostic test for the diagnosis of malaria in Jimma, southwestern Ethiopia

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ABSTRACT

Objective: To evaluate the diagnostic performance of CareStart™ Malaria Pf/Pv Combo test relative to microscopy, for the diagnosis of *falciparum* and *vivax* malaria in Ethiopia.
Methods: Two hundred and forty febrile patients visiting the Serbo health center in Jimma zone, southwestern Ethiopia, were involved in this study in 2008. Giemsa-stained thin and thick blood smears were prepared and microscopically examined under a 100× oil immersion microscope objective for *Plasmodium* species identification and determination of parasitemia respectively. CareStart™ Malaria Pf/Pv Combo test was performed as per the manufacturers' instruction.
Findings: The validity of CareStart™ Malaria Pf/Pv Combo test for the diagnosis of *Plasmodium* was very good with a sensitivity of 95.8%, specificity of 100%, positive predictive value of 100% and negative predictive value of 96%. The test performed equally well for the identification of *Plasmodium falciparum* and *P. vivax*. The diagnostic performance of this CareStart™ test is comparable to light microscopy of thin and thick blood smears.
Conclusion: Although CareStart™ Malaria Pf/Pv Combo test and blood microscopy have comparable diagnostic performance for *Plasmodium* detection, the CareStart™ test has the added advantage of being simple to interpret, cost-efficient, and hence it is preferable to use this rapid diagnostic test for malaria diagnosis in areas where microscopy is not accessible and during times of malaria epidemics that are observed approximately every 4–5 years in Ethiopia.

1. Introduction

In Ethiopia, malaria is the leading cause of morbidity and mortality. Almost 75% of the country is malarious and an estimated 51 million people (68% of the population) live in areas at risk of malaria. The number of annual malaria cases is as high as 10–15 million (FMOH, 2006) and 60–70% of the cases are attributable to *Plasmodium falciparum* infection while 30–40% of the cases are attributed to *P. vivax* infections (Ghebreyesus et al., 1996; Deressa et al., 2003; Jima et al., 2005). *P. falciparum* is responsible for 13–28% of deaths in children under 5 years of age (Tulu et al., 1993).

The main malaria control strategies in Ethiopia include early case detection and immediate treatment, sustainable vector control and prevention and control of epidemics (WHO, 1993; FMOH, 2004; Adhanom et al., 2006). Early diagnosis of cases is accomplished either through laboratory diagnosis at health centers and hospitals or through clinical diagnosis or Rapid Diagnostic tests (RDTs) at peripheral health facilities where microscopy is not available (FMOH, 2004).

Microscopic examination of Giemsa-stained thick and thin blood films has remained the gold standard technique for malaria diagnosis (Anthony, 2002). However, it is difficult to maintain good microscopy at peripheral health care services where most patients are treated (WHO, 2000). Most cases of fevers are treated presumptively as malaria without laboratory-confirmed diagnosis. The Federal Ministry of Health (FMOH) of Ethiopia introduced Artemisinin combination therapy (ACT) as the first line drug for the treatment of uncomplicated falciparum malaria in 2004 (FMOH, 2004). Chloroquine is the first line drug for the treatment of uncomplicated *P. vivax* malaria. This presumptive treatment of all fevers as malaria may result in extensive overuse of antimalarials and delays the diagnosis of other causes of fever (Olivar et al., 1991; Luxemburger et al., 1998; Chandramohan et al., 2002).

Moreover the diagnosis of malaria by microscopy of Giemsa-stained blood smears is a labor-intensive method that requires quality staining, precision microscopes, trained and experienced microscopists. These limitations have prompted research and development of simple, RDTs to detect the presence of malaria

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parasites at levels of accuracy comparable to those of a skilled microscopist. Currently, malaria RDTs that identify circulating antigens of malaria parasites are being used as alternatives for presumptive case management (Guthmann et al., 2002; Hopkins et al., 2007).

RDTs for malaria diagnosis are also introduced at health facilities where microscopy is not available, for proper use of the drug for falciparum malaria and to avoid drug wastage as the drug is expensive. Assays are based on the capture of parasite antigen by monoclonal antibodies incorporated into a test strip. Three types of antigens are targeted: parasite lactate dehydrogenase (pLDH), histidine-rich protein 2 (HRP-2, found in *P. falciparum* only) and aldolase (pan-malarial antigen, found in all malarial species). HRP-2 or *P. falciparum*-specific pLDH assays are often combined with pan-specific pLDH or aldolase antigen assays in tests that can differentiate *falciparum* malaria (if the HRP-2 and pan-specific bands are positive) from non-*falciparum* malaria (if the pan-specific band only is positive). Some tests include pLDH antibodies for *P. vivax*-specific pLDH (Allen, 2006).

Although there has been a marked increase in the number of commercially available RDTs, they can be roughly divided into two categories. One group of RDTs, detect the histidine rich protein 2 (HRP2), a protein uniquely synthesized by *P. falciparum* and circulating in the infected individual's bloodstream, and this RDT is termed Paracheck-Pf®. This RDT is currently in use mainly in peripheral health facilities, however has the disadvantage of only diagnosing *P. falciparum*, but not the other *Plasmodium* species, particularly *P. vivax* that is prevalent in Ethiopia. The FMOH made the choice of procuring and using these RDTs due to their low cost and their availability on the market. To improve the quality of malaria diagnosis and treatment at peripheral health facilities (health posts), the availability of RDTs that can confirm *Plasmodium* etiology is crucial. In new RDTs, the HRP2 test kits are designed also to detect aldolase or parasite lactate dehydrogenase (pLDH), proteins synthesized by all four human-infecting *Plasmodium* species (Swarthout et al., 2007).

Now that cheaper and more user-friendly multispecies RDTs are available on the market, the FMOH has revised the product selection and it is now anticipating to procure such RDTs that will enable more specific diagnosis of malaria etiology, resulting in better malaria case management. In addition, the use of these RDTs will help establishing the country's distribution of *Plasmodium* species, thereby also improving the understanding of the epidemiology of the disease. The use of multispecies RDT also encourages local health workers (health extension workers) to treat fever cases properly. Thus, RDTs that detect both *P. falciparum* and *P. vivax* are important in peripheral health care systems of the country as proper diagnosis of malaria cases is very crucial for cost-effective treatment and sustainable use of artemether–lumefantrine.

Our present study evaluated the diagnostic performance of a recently developed RDT, the CareStart™ Malaria Pf/Pv Combo test for the diagnosis of malaria due to *P. falciparum* and *P. vivax* relative to microscopy in Jimma zone, southwestern Ethiopia, where both malarial parasites are prevalent.

2. Materials and methods

2.1. Study area and subjects

Diagnostic performance of CareStart™ Malaria Pf/Pv Combo test (detects HRP2 antigen and pLDH) as compared to Giemsa-stained blood smear microscopy was evaluated at the Serbo health center in Jimma zone, southwestern Ethiopia, from October 2007 to December 2008. This center is located about 300 km South west of Addis Ababa, and is situated 1760 m above sea level. Malaria transmission takes place throughout the year. The study subjects recruited in Serbo were 240 febrile patients, clinically suspected for malaria, who visited outpatient department (OPD) of the health center.

The CareStart™ Malaria Pf/Pv Combo test and microscopic examination of Giemsa-stained blood smear were performed on each of the 240 febrile patients. After informed consent was obtained, venous blood was drawn into EDTA-filled tubes to be used for routine examination for malaria, including automated complete blood counts.

2.2. Microscopic examination

Thick and thin blood smears were prepared on a slide, stained with Giemsa, and examined under a 100× oil immersion microscope objective. Parasitemia was determined from thick smear while *Plasmodium* species identification was done from the thin smear. Three experienced technicians examined the slides independently. The technicians examined at least 300 high-power fields in thin smears before classifying the slides as being negative.

2.3. CareStart™ Malaria Pf/Pv Combo test

Each CareStart™ Malaria Pf/Pv Combo rapid diagnostic test device was given an identification code similar to the code used on the slide for each study subject. Then, 5 μl of fresh blood sample was added onto the test device window using the sample applicator provided with the kit, followed by adding 4 drops of reagent buffer. The device was then left aside for 20 min at room temperature and the result was recorded as per the instruction of the manufacturer (Access Bio Inc., Monmouth Junction, New Jersey, USA). The results were recorded as negative if only one pink color band appeared on the control area whereas the presence of two bands, one band in the control area and the other band in the test area (T1, corresponding to *P.f.* HRP2) was recorded as a positive result for *P. falciparum* and similarly one band in the control area and the other band in the test area (T2, corresponding to pLDH) were recorded as a positive result for *P. vivax*. Mixed infections for *P. falciparum* and *P. vivax* were recorded when a control band and two test area bands appeared simultaneously. The test was considered invalid when no control band appeared. *P. malariae* and *P. ovale* were not considered during evaluation of the CareStart™ Malaria Pf/Pv Combo test because the occurrence of these species in Ethiopia is insignificant. *P. malariae* accounts only for less than 1% of cases and is restricted in its distribution while *P. ovale* is rarely reported (CNHDE, 2007).

2.4. Quality control

Test kits were stored as per the directions of the manufacturer and quality of package desiccant also checked before use. Fresh blood samples were transferred directly to the sample pad by the provided sample applicator. All CareStart™ malaria tests were labeled with patient ID number and result recorded 20 min after adding 4 drops of clearing buffer. In all cases, the results of the CareStart™ test were determined prior to microscopic results with strict blinding to microscopic examination of the blood film.

To eliminate observer bias, three experienced malaria technicians performed the microscopic examination of the Giemsa-stained blood film and the CareStart™ test independently. The results of their observation were recorded for later comparison on separate sheets and a quality control was done by repeating all discordant results between the RDTs and the slides.

2.5. Ethical considerations

The study was part of a malaria project funded by Jimma University, and institutional ethical approval from the ethical clearance

Table 1
The comparison of Care Start™ malaria RDT to microscopy.

Care Start™	Microscopy		Total No. (%)
	Positive, No. (%)	Negative, No. (%)	
Positive	115 (95.8)	0 (0)	115 (47.9)
Negative	5 (4.2)	120 (100.0)	125 (52.1)
Total	120(100.0)	120(100.0)	240

committee of the Jimma Univ. Research Council was obtained. Although the study subjects were symptomatic patients who presented on their own initiative to the health center, verbal consent was obtained from each participant and guardian in case of children under 18 after clear explanation of the study objective.

2.6. Data analysis

The data were entered using EXCEL and analyzed using SPSS Version 16.0. Sensitivity, specificity and predictive values of the assay were calculated for the CareStart™ malaria test using microscopy as the gold standard. The following equations were used in calculating sensitivity, specificity and predictive values of the assay:

$$\text{sensitivity} = a/n1 \times 100$$
$$\text{specificity} = b/n2 \times 100$$
$$\text{positive predictive value} = a/n3 \times 100$$
$$\text{negative predictive value} = b/n4 \times 100$$

where $n1$ = number of true positives identified by microscopy;
$n2$ = number of true negatives confirmed through microscopy;
$n3$ = number of cases identified as positives by a diagnostic test;
$n4$ = number of cases identified as negatives by the diagnostic test;
a = number of cases identified as positives by microscopy and by the diagnostic test;
b = number of cases identified as negatives by microscopy and by the diagnostic test.

3. Results

In this study a total of 240 patients were included. The patients ranged in age from 1 to 60 years, with a mean age of 25 years. The male to female ratio of the participants were 1.38:1. Parasite positivity in both sexes was 57.5% men versus 42.5% women. From the total study participants 134 (55.8%) were from rural area and the rest 106 (44.2%) were from urban area. Concerning educational status, 88 (36.7%) were illiterate and the rest 152 (63.3%) were found to be literate. In this category volunteers with high school/university education displayed lowest parasite positivity levels (6%).

The microscopic examination result indicated that 120 patients were positive for malaria with different parasite densities. The rest were negative by microscopy. From the total positives scored by microscopy, 56(23.3%) were *P. falciparum* and the rest 64(26.7%) were *P. vivax*. Care Start™ malaria RDT was evaluated against Giemsa stained microscopy. As compared to microscopy, Care Start™ malaria RDT detected malaria in 115 (47.9%) patients, and detected no infection in the rest 125 (52.1%) (Table 1).

Table 3
Identification of the two *Plasmodium* species by Care Start™ RDT versus microscopy.

Care Start™	Microscopy		Total No. (%)
	P. falciparum, No. (%)	*P. vivax*, No. (%)	
P. falciparum	54 (96.4)	0 (0.0%)	54 (45.0%)
P. vivax	0 (0.0)	61 (95.3%)	61 (50.8%)
Negative	2 (3.6%)	3 (4.7%)	5 (4.2%)
Total	56 (100.0)	64 (100.0)	120 (100.0)

Table 2 depicts the range of sensitivity, specificity, PPV and NPV during detection of each parasite species by RDT relative to microscopy. The sensitivity of the test to various parasite densities was consistently high; patients detected positive for malaria by the RDT suffered different parasite densities ranging from 960 parasites/μL to 3860 parasites/μL. From the total 120 malaria patients detected by microscopy, Care Start™ malaria RDT identified 54 (45%) as *P. falciparum* and the rest 61 (50.8%) were identified as *P. vivax*. In contrast, Care Start™ RDT failed to detect 2 (3.6%) *P. falciparum* and 3 (4.7%) *P. vivax* infections (Table 3). In these 5 cases that reported parasitemia below 100 parasites/μL, the parasite densities were reported to be 96, 96, 98, 96 and 98 parasites/μL respectively.

4. Discussion

About 40,000 Ethiopians die from malaria every year, more than those dying from HIV and tuberculosis. On top of the yearly peak in malaria cases during Ethiopia's rainy season, malaria epidemics occur every three to four years. In 2003, the usual 6 million cases shot up to 16 million, and over 100,000 people died. Malaria incidences also vary according to differences in the terrain and climate across the country, as these affect mosquito numbers. While the government has health posts in all rural areas, each covering around 1000 households, there is currently no way of telling where the biggest outbreaks are likely to occur at a particular time, or when they will lead to an all-out epidemic.

The malaria surveillance system, to function properly, requires charts for recording malaria cases. To help meet the need for personnel, the MoH has directed the training of 30,000 health workers, with at least two women per health post, to liaise with locals and educate them about the risks and treatments. Coartem, an artemisinin-based therapy is recommended by the WHO as the first-line treatment for malaria in Ethiopia, with the goal to provide prompt and effective treatment to 60% of the population at risk of malaria within 24 h of the onset of fever, and thereby halve its malaria burden by 2010. Accurate diagnosis is however critical for treatment to be cost-effective. Coartem, given to every *P. falciparum* infected patient costs US$2 per treatment. *P. vivax*, which causes about 40 per cent of malaria cases in Ethiopia, is treatable with chloroquine, at only 50 cents per treatment. The gold standard has been to use microscopy to detect malaria that requires trained personnel and equipment. UNICEF is currently attempting to boost the availability of microscopes in district-level health clinics, yet this will not meet the demand at smaller, more numerous, rural health posts.

Table 2
Diagnostic performance of CareStart™ Malaria Pf/Pv Combo test relative to microscopy in the detection of *Plasmodium* species, in febrile patients at Serbo health center, Jimma zone, Ethiopia 2008.

RDT	Parasite	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
CareStart™	*Plasmodium*	95.8 (90–97)	100	100	96 (95–98)
	P. falciparum	96.4 (95–98)	100	100	96.9 (95–99)
	P. vivax	95.3 (90–97)	100	100	94.9 (90–97)

CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value.

Rapid diagnostic test (RDT) use can overcome shortages in staff and equipment. The small packs are easy to transport and operate, and when stored properly, guarantee specificity and sensitivity. Each RDT costs 60 cents, thus is cost-effective, allowing Coartem to be used for only the cases that require it. The CareStart™ Malaria Pf/Pv Combo RDT revealed a high level of sensitivity (>95%) and specificity (>99%), thus fulfilling the performance criteria set for the rapid diagnosis of malaria (WHO, 2003; Mendiratta et al., 2006). On the other hand, we observe that the sensitivity of the test is low when the density is below 100 parasites/μl of blood. Such observations have also been reported previously, mostly in asymptomatic cases (Kaklciaya, 2003). This indicates the need for further evaluation of the test on malaria patients with low parasitemia.

The specificity of CareStart™ malaria test observed presently for diagnosing *P. falciparum* and *P. vivax* was also relatively higher than the specificity reported by Fogg et al. (2008) and Ratsimbasoa et al. (2007). Such variations with the same test can be explained by the persistence of *P.falciparum* specific HRP2 in patients who had been treated a month ago (Kaklciaya, 2003; Guthmann et al., 2002).

CareStart™ RDT is preferable to other tests like the Paracheck Pf® test, as it has the advantage of differentiating *P. falciparum* from *P. vivax*. This is very important for treatment decisions at all health facility levels in general and at peripheral areas in particular where there is no access to microscopy. Such targeted treatment saves unnecessary consequences that result when treatment is based on clinical symptoms alone. Furthermore, CareStart™ Malaria Pf/Pv Combo test can be used to follow drug treatment. Dead parasites no longer generate parasite-specific LDH, thus subsequent tests would be negative.

Although light microscopy often remains the method of choice for diagnosing malaria in a clinical laboratory, it is operator dependent, time consuming and requires regular examination of several fields to maintain competency. During parasite asexual reproduction in deep capillaries, or parasite sequestration, *Plasmodium* can be difficult to enumerate by microscopy of peripheral blood sample. In such limiting situations, the RDTs are an efficient alternative. Expertise and proficiency required to carry out these tests and interpret results are less strict as compared to microscopy.

Similar studies have been performed with the OptiMAL® dipstick in malaria diagnosis during pregnancy (Tagbor et al., 2008). Malaria infection of the placenta may occur in the absence of peripheral blood parasitemia, and in women with a positive OptiMAL® test but a negative blood film, the RDT detects a placental malaria infection. Studies of the use of rapid diagnostic tests in pregnant women have been undertaken mainly for the purpose of diagnosing placental malaria at delivery, and such studies report the tests as having good diagnostic features.

In our hands, the Care start™ showed an overall sensitivity, specificity, PPV, and NPV of 95.8%, 100%, 100%, and 96% respectively. This RDT thus shows a better efficacy than other RDTs used in previous studies (Gatti et al., 2002), or even in Ethiopia using immunochromatographic antigen detection assays (Girum and Girma, 2001). In our hands, the Care start™ RDT displayed good test efficiency and reliability in diagnosing malaria. We recommend the active use of Care start™ RDT as an effective epidemiological tool for the rapid screening of malaria in Ethiopia.

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