Original article

Performance of CareStart™ Malaria Pf/Pv Combo test for the diagnosis of *Plasmodium falciparum* and *Plasmodium vivax* infections in the Afar Region, North East Ethiopia

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Abstract

Background: CareStart™ Malaria Pf/Pv Combo test has shown encouraging results for the diagnosis of *P. falciparum* and *P. vivax* infections in Ethiopia. Nevertheless, the performance of the test could be affected by different factors like gene polymorphisms, excess heat and humidity. Hence, evaluation of the performance of the test in different settings in Ethiopia is vital for the routine diagnosis of malaria.

Objective: To evaluate the diagnostic performance of CareStart™ Malaria Pf/Pv Combo test for the diagnosis of *P. falciparum* and *P. vivax* infections in the Afar Region, Northeast Ethiopia.

Methods: Finger prick blood samples were collected from a total of 1092 patients who had malaria symptoms and visited three different health facilities in the Afar Region. Giemsa-stained thin and thick blood smears were prepared and microscopically examined under 100 × magnifications for *Plasmodium* species identification and determination of parasitaemia. CareStart Malaria Pf/Pv Combo test was also performed as per the manufacturer's instructions. The sensitivity and the specificity of the test was determined using microscopy as gold standard.

Results: The sensitivity and specificity of the test were 98.5% and 98.0% respectively, with a positive predictive value (PPV) of 91.7% and a negative predictive value (NPV) of 99.7% for the diagnosis of *P. falciparum* infection. The corresponding sensitivity and specificity for the diagnosis of *P. vivax* infection were 100% and 99.6% respectively, with PPV and NPV of 86.2% and 100%, respectively.

Conclusion: The results of this study revealed high sensitivity and specificity of CareStart™ Malaria Pf/Pv Combo test for the diagnosis of both P. *falciparum* and *P. vivax* infections in the study area, though additional study may be needed in the most peripheral hottest areas of the region. [*Ethiop. J. Health Dev.* 2011;25(3):206-211]

Background

Malaria affects approximately 300-400 million people and causes 1-2 million deaths every year worldwide (1). The disease remains endemic in 109 countries, of which 45 countries are in Africa (2). In Ethiopia, About 75% of the total area of the country is estimated to be malarious, while more than two thirds of the total population is at risk of infection (3). The annual number of malaria cases in Ethiopia is as high as 10-15 million (4) and mainly caused by infection with *P. falciparum* and *P. vivax.*

Accurate and rapid diagnosis of malaria is not only the cornerstone for preventing morbidity and mortality, but also for avoiding unnecessary use of anti-malarial drugs. In many settings in Africa, presumptive treatment of all fevers as malaria results in extensive overuse of anti-malarial drugs and delays the diagnosis of other causes of fever (5). Light microscopy is presently the gold standard for malaria diagnosis. However, its low sensitivity in the case of low level of parasitemia, especially in patients infected with *P. falciparum* (6) necessitates other effective and rapid diagnostic tests.

Rapid diagnostic tests (RDTs) for the diagnosis of malaria have been developed and recognized by WHO as potential solutions to overcome the limitations with microscopy (7, 8). Using RDTs have several advantages particularly in peripheral health facilities such as health posts. They: require no capital investment or electricity, are simple to perform, and are easy to interpret. Users such as community health workers (at health posts) with little or no laboratory experience can be quickly trained in their use, result is available within a short time thus accelerating case-management and can fill diagnostic gaps at health facilities where microscopy is not available and the like.

However, RDTs also have some disadvantages: not possible to quantify the parasitic load and able to assess anti-malaria treatment. This might be because of the false positivity due to the persistence of HRP 2 antigen in patients who had been treated for one month in case of *P. falciparum* (9).

Among commercially available RDTs, CareStart™ Malaria test has been showing encouraging results for the diagnosis of the various species of *Plasmodium* parasites that infect humans in various countries (10, 11). For instance, CareStart Pf/Pv Combo test fulfills the performance criteria set for the rapid diagnosis of malaria (12), by attaining sensitivity greater than 95% for samples with parasitemia≥100 parasites/µl of blood.

RDTs employ lateral-flow immune-chromatographic technology. Whereby, the clinical sample migrates as a liquid across the surface of a nitrocellulose membrane by means of capillary action (13, 14). For a targeted parasite

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antigen, two sets of antibodies, a capture and detection antibodies, are used. The capture antibodies are sprayed as a stripe by machine onto the nitrocellulose membrane and bound to the membrane in an immobile phase. These fixed antibodies serve to extract and bind parasite antigen from the migrating liquid sample. The second set of antibodies is conjugated to an indicator, typically gold particles, in a mobile phase. These antibody-indicator complexes bind to the parasite antigen that has been captured by the immobile antibody on the membrane, producing a visible line if the targeted antigen is present in the clinical sample (8, 14).

In Ethiopia, the performance of CareStart™ Malaria Pf/Pv Combo test, which is based on the detection of HRP-2 antigen for *P. falciparum* and pLDH antigen for *P.vivax* was evaluated in southern Ethiopia. The study showed sensitivity of 99.4%, and specificity of 98% with PPV of 94.4% and NPV of 99.8% for the diagnosis of *P. falciparum* infection. The corresponding value of sensitivity, specificity, PPV and NPV of the test for the diagnosis of *P.vivax* were 99.4%, 98.2%, 94.5% and 99.8%, respectively (15). A similar study showed a sensitivity of 95.8% and specificity of 100% with PPV of 100% and NPV of 96% (16) for the diagnosis of *P. vivax.*

Nevertheless, it is important to evaluate CareStart™ Malaria Pf/Pv Combo test in different geographical areas of Ethiopia since the performance of the test can be affected by different factors like ambient conditions, and variability within the parasite antigen being detected by the RDT (12, 17). Hence, this study was conducted to assess the sensitivity, specificity and predictive values of the CareStart Malaria Pf/Pv Combo test, in patients conformed of malaria using Giemsa stained blood films as reference in the Afar Region, north east Ethiopia. The hypothesis of the study was that CareStart™ Malaria Pf/Pv Combo test would have comparable diagnostic performance with light microscopy for the diagnosis of both *P. falciparum* and *P. vivax* infections with sensitivity and specificity greater than 95%.

Methods

Study Area and Population

A cross-sectional health facility based study was conducted at MelkaWerer Health Centre, Gewane Health Centre and Dubti Hospital of the Afar Region from December 2009 to January 2010. The Afar Region is located in the Great East African Rift Valley in the northeastern part of Ethiopia. The altitude of the region ranges from 1500 meters above sea level to 166 meters below sea level, while the temperature varies from 25ºC during the wet to 48ºC during the dry season. Rainfall is erratic and scarce, and the annual precipitation ranges from 200 mm to 600 mm. Dubti Hospital is located at 574 km from Addis Ababa and it has an average elevation of 503 meters above level, while its annual temperature ranges from 20 ºC to 45 ºC. Melka Werer Health Centre is located at 270 km from Addis Ababa with an average elevation of 740 meters above sea level and its annual temperature ranges from 19 ºC to 34 ºC. Gewane Health Centre which is located at 365 km from Addis Ababa has an average elevation of 550 meters and annual temperature ranging from 33.2 ºC to 42.5 ºC.

The epidemiological pattern of malaria transmission in Ethiopia is generally seasonal and highly unstable due to variations in topography and rainfall patterns. In the Afar Region it is generally unstable, with perennial transmission in areas along the Awash River Valley (18).

The study subjects consisted of all febrile patients who had clinical symptoms of malaria fever of malaria and visited the outpatient department (OPD) of the three health facilities. There were no exclusion criteria unless the patient or the guardians (in case of children less than 18 years old) did not volunteer to participate in the study.

Sample Size Determination

At present, there is no reliable information on the performance characteristics of CareStart™ Malaria Pf/Pv Combo test in the study area. However, the sample size was calculated, based on the sensitivity and specificity of the test to be 95% (12), using the sample size calculation formula $n = (Z/D)^2 \times P(1-P)$,where n = sample size, Z = 95% confidence interval (1.96), D = Margin of error (5%), P = Sensitivity/ Specificity (95%).

Based on this assumption, a minimum number of 83 blood film positive and 83 blood film negative patients (166 for *P. falciparum* and 166 for *P. vivax*) were stipulated to be included in the study for each study area. However, to increase the reliability of the test, more subjects were recruited to participate in the study.

Data Collection

Thick and thin blood films were prepared from finger prick blood, stained with Giemsa for 10 minutes and examined under 1000X magnification. Parasite densities (parasites/µl of blood) were calculated by assuming 8000 WBCs/µl whole blood. Thus, parasites in thick-film fields were counted until 200 WBCs were examined, and the parasite count multiplied by 40 to give the number of parasites per micro liter of blood and the species of *Plasmodium* were identified using thin smear (19). A minimum of one hundred high power fields were examined on a thick film before a negative result was declared (15).

In this study, we used CareStart ™ Malaria Pf/Pv Combo test (Lot No H38 IV and Lot No H28 IV) to screen patients. CareStart ™ Malaria Pf/Pv Combo test (Lot No H28 IV) was stored at ambient temperatures of Dubti Hospital for one year without controlling the system of the storage temperature. The CareStart™ Malaria Pf/Pv Combo test Lot No H38 IV was stored at local temperature in Addis Ababa for about 6 months before starting the study. The CareStart ™ Malaria Pf/Pv Combo test was performed according to the instruction of the manufacturer (Access Bio, Inc., New Jersey, USA).

Briefly, each CareStart™ Malaria Pf/Pv Combo test device was given an identification code similar to the code used on the slide for each study subject. About 5 µl of fresh blood sample was added on the test device window using the sample applicator provided with the kit and followed by adding 3 drops (60 µl) of reagent buffer. The device was left aside for 20 minutes at room temperature and the results were recorded as positive, negative or invalid.

Quality Control

Test kits were kept at the local temperature of the region without any controlling system of the storage temperature during data collection. Quality of package desiccant was checked before use. The results of the RDTs and microscopy examination were recorded on separate sheets with similar identification code for each study subject. To minimize observational bias, experienced malaria technicians performed the microscopic examination of the giemsa stained blood film and the CareStart™ pf/pv malaria Combo test independently. The results of their observation were recorded for later comparison on separate sheets to ensure accuracy. Moreover, at the end of the study, 20% of the positive and 10% of the negative slides and discordant results between CareStart™ Malaria Pf/Pv Combo test and those of microscopy were examined by another well experienced technician. Data collection, examination and recording were supervised by the investigators.

Ethical Considerations

The study protocol was approved by the Ethical Clearance Committee of the Aklilu Lemma Institute of Patho-biology, Addis Ababa University. The aim of the study was explained to each participant and their verbal consent was obtained. Study participants, who were found positive for *P. falciparum*, were treated with an Artemisinin combination therapy (ACT), while those positive for *P. vivax* were treated with chloroquine according to the treatment protocol of the National Guideline.

Data analysis

The data were entered using EpiData Version 3.1 and analyzed using Stata 8 software. Sensitivity, specificity and predictive values of the CareStart™ malaria test were calculated using microscopy as the gold standard. The following equations were used to calculate the sensitivity, specificity and predictive values of the assay (16).

Sensitivity = $a/n_1 \times 100$
Specificity = $b/n_2 \times 100$
Positive predictive value = $a/n_3 \times 100$
Negative predictive value = $b/n_4 \times 100$

where n_1 = number of true positives identified by microscopy; n_2 = number of true negatives confirmed through microscopy; n_3 = number of cases identified as positives by RDT test; n_4 = number of cases identified as negatives by the RDT test; a = number of cases identified as positives by microscopy and by the RDT test; b = number of cases identified as negatives by microscopy and by the RDT test.

Results

A total of 1092 patients suspected of malaria were screened by microscopy and the CarStart™ malaria Combo test. Of those, 675 (61.81%), 238 (21.8%) and 179 (16.4%) were from Melkawerer, Gewane Health Centers and Dubti Hospital, respectively. Of the total participants, 527 (48.57%) were females and 558 (51.43%) males with ages ranging from 3 months to 78 years old (mean ± SD age = 22.3 ± 12.8 years). The gender of 7 individuals was not recorded. One hundred and six subjects (12.5%) had anti-malarial therapy in the preceding one month. Table 1 shows the socio-demographic and baseline characteristics of the study subjects.

Table 1: **Socio-demographic and baseline characteristics of febrile cases suspected for malaria Afar Region, Northeastern Ethiopia, December 2009 to January 2010**

Characteristics	Number (%)
Gender	
Male	558 (51..43)
Female	527 (48.57)
ND	
	7 (0.64%)
Age (year)	
<5yrs	170 (15.68)
5 - 10 yrs	84 (7.69)
11 - 15yrs	86 (7.88)
>15 yrs	752 (68.9)
Treatment history	
Yes	136 (12.5)
No	956 (87.6%)

ND = Not determined

Microscopic blood film examination indicated 201 (18.4 %), 23 (2.1%) and 2 (0.2%) of the total study subjects as positive for *P. falciparum*, *P. vivax* and mixed infections, respectively. On the other hand, 212 (19.4%), 27 (2.5%) and 4 (0.4%) were positive for *P. falciparum, P. vivax,* and mixed infections, respectively, by the CareStart™ combo test. Out of the 201 subjects who were positive for *P. falciparum* infection by microscopy, 196 (97.5%), 2 (0.99 %) and 3 (1.5%) were found positive for *P. falciparum*, *P. vivax* and negative, respectively by the Care Starttest™. All the 23 subjects found positive for *P. vivax* by microscopy were also found positive for *P. vivax* infection by CareStart™ test (Table 2). Again 18 and 4 subjects, who were found negative by microscopy for *P. falciparum* and *P. vivax* infections, respectively, were found to be positive by the CareStart™. Of those, 7 cases had history of malaria treatment within one month prior to the study.

Eighty patients were screened by CareStart™ (Lot No H28IV) which was stored at the ambient temperature of

Afar Region parallel with CareStart™ (Lot No H 38IV) which was stored at Addis Ababa. Similar results were obtained by both tests (Table 3).

Table 4 depicts the sensitivity and specificity of the CareStart™ test for the diagnosis of *P. falciparum* and *P. vivax* infections. The sensitivity and specificity of the test were 98.5% and 98.0% respectively, with PPV of 91.7% and NPV of 99.7%. The corresponding sensitivity and specificity for the diagnosis of *P. vivax* infection were 100% and 99.6%, respectively, with PPV and NPV of 86.2% and 100%, respectively.

As expected, CareStart™ test for the detection of *P. falciparum* had a sensitivity of 100% for samples with parasitemias > 100 parasites/µl and its sensitivity declined lower than 62.5% for samples with parasitemia < 100. Table 5 depicts the sensitivity of CareStart™ Malaria *PF/Pv* Combo test for the diagnosis of *P. falciparum* infections at different levels of parasitemia. Regarding each study site, the sensitivities were 100%, 100% and 98.4% at Dubti Hospital, Gewane and Melkawerer health centres, respectively. The specificities were 98.2% at Dubti, 100% at Gewane and 96.6% at Melkawerer.

Table 2: **Performance of CareStart ™ Malaria Pf/Pv Combo Test for the Diagnosis of *P. falciparum* and *P. vivax* Infections in the Afar Region, Northeastern Ethiopia, December 2009 to January 2010**

Microscopic Results	No samples	CareStart Pf/Pv results			
		P. falciparum	*P. vivax*	Mixed	Negative
P.falciparum	201	196	2	0	3
P.vivax	23	0	23	0	0
Mixed	2	0	0	2	0
Negative	866	16	2	2	846
Total	1092	212	27	4	849

Table 3: **Comparison of CareStart ™ Malaria Pf/Pv Combo Test (Lot No H28IV) and CareStart ™ Malaria Pf/Pv Combo Test (Lot No H38IV) for the Diagnosis of *P. falciparum* and *P.vivax* Infections in the Afar Region, Northeastern Ethiopia, December 2009 to January 2010**

Plasmodium spp.	CareStart™ Pf/Pv (Lot No H38V)	CareStart™ Pf/Pv (Lot No H28IV)	Microscopy
P. falciparum	29	29	29
P. vivax	9	9	9
Mixed	2	2	2
Negative	40	40	40
Total	80	80	80

Table 4: **Sensitivity and Specificity of CareStart ™ Malaria Pf/Pv Combo Test for the Diagnosis of *P. falciparum* and *P. vivax* Infections in the Afar Region, Northeastern Ethiopia, December 2009 to January 2010**

CareStart™		Microscopy			
		Positive	Negative	Sensitivity %	Specificity %
	Positive	198	18	98.5	98.0
P. falciparum	Negative	3	873		
	Positive	23	4	100	99.6
P. vivax	Negative	0	1065		

Table 5: **Over all Sensitivity of CareStart™ Malaria *PF/Pv* Combo Test for the Detection of *P. falciparum* at Different Levels of Parasitemia in the Afar Region, Northeastern Ethiopia, December 2009 to January 2010**

Parasitemia (No of parasite /µl of blood)	Microscopy (No of positive)	CareStart™ (No of positive)	Sensitivity (%)
	201	198	98.5[a]
>5,000	ND	ND	ND
1001 - 5,000	32	32	100
501 - 1,000	108	108	100
100 – 500	53	53	100
< 100	8	5	62.5

ND - No data, a- over all sensitivity

Discussion

In this study, the performance of the CareStartTM was found to be highly sensitive for the detection of both *P. falciparum* and *P. vivax* infections as previously reported from other parts of Ethiopia (15, 16), Uganda (20) and Madagascar (10). Recent studies have shown that its sensitivity is low when the parasite density is less than 100 parasites/µl of blood (16, 21). In this study, three false-negative results were found for *P. falciparum* infection in individuals having parasite density less than 100 parasites/µl of blood. In addition to the low parasite density, factors like poor flow characteristics, monoclonal antibody failure (8, 22) and variation in the number of epitopes present in a particular parasite isolate could also be responsible for the false negative results.

The specificities of CareStart™ observed here for the diagnosis of *P. falciparum* (98.0%) and *P. vivax* (99.6%) infections are comparable with previous findings by Sheraw et al. (15) and Mekonene et al.(16), but higher than the specificities reported by Ratsimbasoa et al. (10) and Fogg et al. (20). False positivity could be due to the persistence of HRP2 antigen in patients who had been treated in the preceding 30 days in case of *P. falciparum* (9). Furthermore, occasional false-positive results because of rheumatoid factors have previously been reported with diagnostic devices based on the detection of HRP 2 (13, 23). On the other hand, sequestration may also reduce the number of circulating parasites to below the microscopic threshold detection level of approximately 50 parasites /µl of blood at the time of blood collection which might result in false negative detection (9, 10).

CareStart ™ Malaria Pf/Pv Combo test (Lot No H28IV which was stored at the surrounding temperature of the Afar Region at Dubti Hospital for one year duration) and CareStart ™ Malaria Pf/Pv Combo test (Lot No H 38IV) which was stored at Addis Ababa, revealed similar results (Table 3). This also indicates that the high temperature of the study area might not have significant impact on the test performance of the Care Start. Hence, the test fulfills the performance criteria set for the rapid diagnosis of malaria (12, 24).

Conclusion

CareStart™ Malaria Pf/Pv Combo test showed a comparable diagnostic performance with blood film microscopic examination results for the diagnosis of *P. falciparum and P. vivax* infections. It has the added advantage of being simple to process, interpret, and quick to use as a diagnostic assay. Hence, it can be used in rural health service facilities where laboratory equipment, electricity, and skilled microscopists are scarce, as is the case in the Afar Region. Further studies may be needed to evaluate the performance of the test in the hottest areas of the Region.

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