Am. J. Trop. Med. Hyg., 90(6), 2014, pp. 1109–1112
doi:10.4269/ajtmh.13-0607

Assessing the Performance of CareStart Malaria *Pf/Pv* Combo Test Against Thick Blood Film in the Diagnosis of Malaria in Northwest Ethiopia

Tadesse Hailu* and Tadesse Kebede

Department of Microbiology, Immunology and Parasitology, College of Medicine and Health Science, Bahir Dar University, Bahir Dar, Ethiopia; Department of Microbiology, Immunology and Parasitology (DMIP), School of Medicine, Addis Ababa University, Addis Ababa, Ethiopia

Abstract. Bivalent rapid diagnostic tests are promising diagnostic tools for *Plasmodium falciparum* and *P. vivax*. Their diagnostic performance was evaluated against thick blood smear to assist national malaria control programs. A cross-sectional study was conducted to evaluate the performance of CareStart against thick blood smears among 398 acute febrile patients visiting the Felegeselam Health Center in December of 2011. Thick blood smears were examined under 100× objectives to diagnose *Plasmodium* species. Similarly, CareStart Malaria *Pf/Pv* Combo Test was performed as per the manufacturer's instruction. The ability of CareStart Malaria *Pf/Pv* Combo Test to diagnose *Plasmodium* malaria was very good, with 99.8% (95% confidence interval = 97.7–100%) sensitivity and 97.7% (95% confidence interval = 94.6–99.1%) specificity. The sensitivity and specificity of the CareStart Test is comparable with the thick blood smear in diagnosing malaria. Hence, it is preferable to use the CareStart Malaria *Pf/Pv* Combo Test instead of microscopy in areas where microscopic diagnosis is limited.

INTRODUCTION

Malaria is the world's most important parasitic disease, and it is accountable for the death of more people. It is a major cause of morbidity and mortality among acute febrile illnesses (AFIs) in developing countries in the tropical and subtropical regions of the world. Approximately 3.3 billion people live in malaria areas; malaria affects 300–500 million people annually and is estimated to kill approximately 1–3 million people each year throughout the world. Of these deaths, 90% occur in African children, especially in sub-Saharan Africa.[1,2] Nearly 1 million children under 5 years of age die of malaria every year.[3,4] Most deaths occur in children in rural areas of the continent because of a lack of effective diagnosis and treatment.[5] Therefore, proper case identification and management of malaria within the first 24 hours of onset are considered to be the best ways to reduce its morbidity and mortality.[6]

A previous report in Ethiopia showed that malaria is still the leading cause of outpatient visits, accounting for 12% of cases,[7] but the number of malaria cases reported by health facilities tells us only a portion of the actual magnitude of malaria cases because of low service use rate.[8] However, a recent report indicated that the numbers of malaria admissions and deaths are decreasing because of prevention, diagnosis, and treatment tasks done in the country.[9]

Diagnosis of malaria based on clinical signs and symptoms alone is not specific and usually leads to excessive use of antimalarial drugs.[3] As a result, the World Health Organization recommends that malaria should be confirmed by parasite-based diagnosis before treatment is given. Severe malaria disease needs to be confirmed by parasitological examination of the presence of falciparum malaria by either thick blood smear or rapid diagnostic tests (RDTs),[9,10] although their sensitivity depends on parasitic density.[11] Thick blood smear microscopic examination is the gold standard diagnostic tool for both severe and uncomplicated malaria in many areas of sub-Saharan Africa, but its accessibility is limited.[6] In areas where laboratory-based diagnostic service is not available, clinical signs and symptoms and use of RDTs are alternative approaches that could be adopted until a time when microscopic diagnostic services expand.[3]

In Ethiopia, thick blood smear microscopic diagnosis of malaria is only available at health centers, hospitals, and higher private health facilities but absent in health posts. Thick blood smear microscopy can be more sensitive (detecting as few as 20 parasites/μL blood)[9] than RDT (detecting as few as 100 parasites/μL).[12] In 2005, single species RDTs were introduced at health posts in Ethiopia, greatly improving the accurate diagnosis of *Plasmodium falciparum* malaria at peripheral levels.[8] However, at the moment, multispecies RDTs, like CareStart Malaria *Pf/Pv* Combo Test, which is capable of specifically detecting both *P. falciparum* and *P. vivax* species, are being supplied by the Ethiopian Ministry of Health to health posts. This test enhances malaria diagnosis by the species level at the periphery health posts. Furthermore, it reduces the difficulty of empiric treatment and the wastage of antimalarial drugs.[9]

CareStart is a very crucial means of diagnosis to implement cost-effective treatment and sustainable use of artemether-lumefantrine. RDTs that detect both *P. falciparum* and *P. vivax* are important in peripheral health care systems of the country, because they can detect malaria at the species level. In addition, the need for well-trained personnel and huge resource investments is minimal. In line with this finding, we conducted this study to evaluate the diagnostic performance of the CareStart Malaria *Pf/Pv* Combo Test for the diagnosis of malaria relative to thick blood smear microscopy in northwest Ethiopia, where *P. falciparum* and *P. vivax* are coendemic. Therefore, timely evaluating the performance of these RDTs relative to the gold standard is very important.

MATERIALS AND METHODS

A cross-sectional study was conducted in Pawe Special Woreda among acute febrile patients who attended the Felegeselam Health Center in December of 2011. The study area is located at a distance of 570 km northwest of Addis Ababa. The area has an elevation between 1,000 and 1,050 m

*Address correspondence to Tadesse Hailu, Department of Microbiology, Immunology and Parasitology, College of Medicine and Health Science, Bahir Dar University, PO Box 79, Bahir Dar, Ethiopia. E-mail: tadessehailu2005@yahoo.com

above sea level. The annual temperature of the area ranges from 28°C to 43°C, with average annual rainfalls of 1,050 mL. It has an estimated total population of 50,307, consisting of 25,323 females and 26,984 males. Malaria transmission takes place throughout the year in the Pawe area, although malaria transmission is seasonal in most areas of Ethiopia.

In total, 398 acute febrile illness patients who visited the outpatient department (OPD) of the health center were selected as study participants. Eligible study subjects were those patients with acute febrile illnesses (body temperature > 37.5°C) or history of fever during the last 2 weeks at the date of data collection. Individuals who took antimalarial drugs within the last 2 weeks before the data collection date or refused participation were excluded from the study. The CareStart Malaria *Pf/Pv* Combo Tests and both thick and thin blood smear microscopic examinations were performed on each of the acute febrile patients. The diagnostic performances of the CareStart Malaria *Pf/Pv* Combo Tests targeting histidin rich protien 2 (HRP2) and *Plasmodium* lactate dehydrogenase (PLDH) antigen tests were evaluated against thick blood smear microscopy.

Training of health staff on how acute febrile cases are selected, collection of samples for laboratory diagnosis, and explanation about the study were given before samples were collected. Written informed consent was obtained from every study participant, including the parents and guardians of children. The blood sample was collected by finger prick from each acute febrile patient using a sterile disposable lancet.

Both thick and thin blood smears were prepared on the same slide. To prepare thick blood smears, three drops of blood were distributed over an area of 1 cm^2 at one end of the slide. Thin blood smears were also done by evenly distributing one drop of blood, which was then fixed with methanol before staining. Labeling the slides with the patients' identification numbers was also done. The smears were air-dried, stained using 6% Giemsa stain solution for 10 minutes, washed with distilled water, and air-dried. Identification of *Plasmodium* and parasite count were done on the thick blood smears with 100× objective. Similarly, thin blood smears were examined under 100× magnification for morphological study and species identification.[8,9,12]

The CareStart Malaria HRP-2/pLDH (*Pf/pan)* Combo Test is a lateral flow antigen detection test in a cassette format (Access Bio, Belgium). It is a three-band RDT targeting HRP-2 and pan pLDH. RDT/CareStart Tests were donated by the Ministry of Health. The quality of the package, the temperature in which the CareStart was stored, and the expiration date were checked before use. Test kits were stored at room temperature and used as a means of diagnosis for malaria according to the directions of the manufacturer. Fresh blood samples of 5 μL were transferred directly to the sample pad by the provided sample applicator. All CareStart Malaria Tests were labeled with a patient identification number, and the results were recorded 20 minutes after adding two drops (60 μL) of clearing buffer. The presence of a unique HRP-2 line indicates an infection with *P. falciparum*, whereas a unique pan-pLDH line is found when infected with one or more of the non-falciparum species. The presence of both HRP-2 and pan-pLDH lines indicates an infection with *P. falciparum* or a mixed infection with *P. falciparum* and one or more of the non-falciparum species. A test result without a control line was considered invalid. Invalid tests were retested by taking fresh blood from each patient who had an invalid test result, and the number of invalid tests was recorded.[13]

In all cases, the results of the CareStart Test were determined earlier than microscopic results, with strict blinding to microscopic examination of the thick blood smears. The independent readings of the thick blood smears determined by the experienced malaria technologists were checked by the principal investigator, who was also experienced. To eliminate observer bias, two experienced malaria technologists performed the microscopic examination of Giemsa-stained thick blood smears blindly and independently. The results of their observations were recorded for later comparison on separate sheets. Quality control was done by repeating all discordant results. The discordant thick blood smears were rechecked by the principal investigator using 100× objective.

Ethical considerations. Ethical clearance was obtained from the Microbiology, Immunology, and Parasitology Department Ethical Review Committee, College of Health Science, Addis Ababa University and the Pawe Special Woreda Health Office. Malaria-positive cases were treated with antimalarial drugs based on the current national treatment guideline of Ethiopia.

Data entry and analysis. Data were entered into Microsoft Excel, checked for correctness, and exported to and analyzed using SPSS, version 16. Sensitivity, specificity, positive predictive values (PPVs), negative predictive values (NPVs), and κ-value of CareStart Malaria Tests were calculated using thick blood smear microscopy as the gold standard.

RESULTS

Three hundred ninety-eight acute febrile cases were examined for malaria parasites by thick blood smear microscopy and CareStart Tests at the Felegeselam Health Center. Of 398 participants, 44.2% were males, and 55.8% were females; the age range was 1–70 years. By thick blood smear microscopy (the gold standard), 201 (50.5%) of 398 patients tested were positive for malaria parasites. Of these patients, 195 (49%) were infected with *P. falciparum*, and 6 (1.5%) were infected with *P. vivax* ($N = 398$). Among the positive cases, *P. falciparum* and *P. vivax* accounted for 97% and 3% of the cases, respectively ($N = 201$). No mixed infection was present in this study (Table 1). Similarly, by the CareStart method, 205 (51.5%) of 398 patients tested were positive for malaria parasites. Among 205 people with malaria, 97.1% of the cases were infected with *P. falciparum*, and the remaining 2.9% were infected with *P. vivax* as detected by CareStart (Table 2).

Taking a thick blood smear as a gold standard test for malaria, the sensitivity and specificity of CareStart RDTs were found to be 99.8% (95% confidence interval [95% CI] = 97.7–100%) and 97.7% (95% CI = 94.6–99.1%), respectively.

TABLE 1
The performance of CareStart against thick blood smears among AFI patients at Felegeselam Health Center, northwest Ethiopia in 2011

	Blood film result		
CareStart	Positive *N* (%)	Negative *N* (%)	Total *N* (%)
Positive	201 (99.8)	4 (2.3)	205 (97.8)
Negative	0	193 (97.7)	193 (99.7)
Total	201	197	398

$P < 0.001$; $\chi^2 = 7.205$; $\kappa = 0.97$.

TABLE 2

Prevalence of malaria among febrile patients visiting Felegeselam Health Center, northwest Ethiopia in 2011

Types of methods	Result			
	Examined *N*	Pf *N* (%)	Pv *N* (%)	Total positive *N* (%)
Thick blood film	398	195 (49)	6 (1.5)	201 (50.5)
CareStart	398	199 (50)	6 (1.5)	205 (51.5)

Pf = *P. falciparum*; Pv = *P. vivax*.

The PPV and the NPV of CareStart were found to be 97.8% (95% CI = 94.8–99.1%) and 99.7% (95% CI = 97.6–100%), respectively (Table 3). Difference in the detection of malaria parasites using either thick blood smear or CareStart RDT was significant ($\chi^2 = 7.205$, $P < 0.001$). There was also an excellent agreement between thick blood smears and CareStart RDT Test results, with a κ-value of 0.97 (Table 1).

The CareStart Test was 99.7% (95% CI = 97.6–100%) sensitive and 97.8% (95% CI = 94.7–99.1%) specific to diagnose *P. falciparum* malaria. The PPV and NPV of CareStart to diagnose *P. falciparum* were also 97.8% and 99.7%, respectively. The corresponding sensitivity and specificity of CareStart for the diagnosis *P. vivax* malaria were 99.9% (95% CI = 98.8–100%) and 99.9% (95% CI = 98.8–100%), respectively, with 92.9% PPV and 99.9% NPV (Table 3).

There were four (1%) discordant results obtained between the thick blood film and CareStart. All the discordant results were negative by thick blood film but positive by CareStart. There were also two (0.5%) invalid test results found by CareStart. The invalid test results were repeated with the same test kits and found to be negative. The parasite load count of each positive case was greater than 100 parasites/μL. There was only one case that had a parasitic load less than 200 parasites/μL. The mean, median, and range of the parasite load count were 11,409, 3,280, and 160–400,000 parasites/μL, respectively.

DISCUSSION

Proper diagnosis of malaria, a key factor in the prevention of malaria transmission, is not easily accessed in developing countries. The problem is significantly higher in remote areas of the tropics. The choice of diagnostic tests that can be done in remote areas is very important. Tests should be tested regularly based on performance and cost-effectiveness. The tests can be done in areas without electricity and well-trained manpower. For instance, RDTs like CareStart help to give appropriate diagnoses and treatments of malaria cases within 24 hours in remote areas without a microscopic facility.

The overall prevalence of malaria in the present study was very high, which was detected by either the thick blood film (50.5%) or CareStart RDT (51.5%). The result was in line with the report from Wondogenet by either thick blood film (47%) or CareStart (49.6%).[14] However, the findings were higher than the report from Kola Diba by either thick blood film (40.9%) or CareStart (39.4%).[15] This difference might be because of the geographical variation of the Pawe area, which has the most malaria cases in Ethiopia and the highest malaria transmission throughout the year. However, it might also be because of the development of antimalarial or insecticide resistance and the knowledge, attitudes, and practice differences of the participants regarding the appropriate use of insecticide-treated bed nets in the area.

The attempt to evaluate the performance of the CareStart Malaria *Pf/Pv* Combo Test to diagnose *Plasmodium* infections revealed a high level of sensitivity (99.8%). The high sensitivity achieved using this CareStart Test was quite comparable with previous CareStart Tests that were reported to have 99.4% in Wondogenet,[14] 95% in Kola Diba,[15] 94.91% in Karachi,[16] 99.4% in Sierra Leone,[17] and 100% in Ghana[18]; however, it was higher than the study reported (89.68%) in China–Myanmar.[19] The higher sensitivity to diagnose *Plasmodium* infections compared with the study reported in China might be because the parasite load found there was greater than 100 parasite/μL. Thus, the CareStart Malaria *Pf/Pv* Combo Test fulfills the performance criteria set for the rapid diagnosis of malaria[9,12,20] (i.e., attains sensitivity greater than 95% for samples with parasitemia ≥ 100 parasites/μL blood).

The sensitivity (99.7%) that we report here for the CareStart Malaria *Pf/Pv* Combo Test to detect *P. falciparum* species was comparable with the sensitivity findings reported as 99.4% in Wondogenet,[14] 98% in Uganda,[21] and 96.91% in Karachi,[16] but our finding was higher than the reports of 88.8% in Belgium,[13] 89.1% in Myanmar,[22] and 88.52% in China–Myanmar.[19] Because the parasite density in the present study was greater than 100 parasites/μL blood, which was shown in the microscopic examination of thick film, the sensitivity was higher. This type of relationship between parasite density and RDT performance has also been indicated earlier,[13] showing that, when the parasite load is > 100 parasites/μL, the performance of CareStart will be high. However, the sensitivity of the RDT is low when the parasite density is below 100 parasites/μL blood (mostly in asymptomatic cases).[9,23] This finding indicates the need for additional evaluation of the test on malaria patients with low parasitemia.

The specificities of the CareStart Malaria Test obtained presently for diagnosing *P. falciparum* (97.8%) and *P. vivax* (99.9%) was also similar to findings in a study of *P. falciparum* (98%) and *P. vivax* (98.2%) in Wondogenet[14] The present study (97.7%) specificity was also comparable with the findings in previous studies in Karachi[16] (96.21%) and Sierra Leone[17] (96%) but relatively higher than the finding in a similar study in Uganda (72%).[21] Such high specificity in the present study might be found with a very sensitive test that is able to detect malaria antigens in the presence of very low parasites that are not detected by microscopy.[24]

Such variations within the same test could be explained by the persistence of HRP2 in patients who had been treated 28 days before in case of *P. falciparum*[12] and the sequestration

TABLE 3

Diagnostic performance of the CareStart Malaria *Pf/Pv* Combo Test relative to thick blood smear microscopy in the detection of *Plasmodium* species at Felegeselam Health Center, northwest Ethiopia, in 2011

Species	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
All species	99.8 (97.7–100)	97.7 (94.6–99.1)	97.8 (94.8–99.1)	99.7 (97.6–100)
Pf	99.7 (97.6–100)	97.8 (94.7–99.1)	97.8 (94.6–99.1)	99.8 (97.7–100)
Pv	99.9 (98.8–100)	99.9 (98.8–100)	92.9 (56.1–99.2)	99.9 (98.8–100)

of the parasites in the deep organs to reduce circulating parasites.[25–27] Sequestration may reduce the number of circulating parasites to below the microscope threshold detection level.[23] RDT-based diagnosis is vital to make treatment decisions at all health facility levels in general and the peripheral areas in particular, where there is no microscopy. Such parasite-based treatment reduces unnecessary consequences resulting from treatments given based on clinical symptoms, including the development of drug resistance. Furthermore, the CareStart Malaria *Pf/Pv* Combo Test could be used to follow drug treatment in areas where there is no microscopic facility. Therefore, the CareStart Malaria *Pf/Pv* Combo Test is a promising device for diagnosis of *P. falciparum* and *P. vivax* (instead of microscopy) in rural areas where the two species are coendemic.

CONCLUSION

The CareStart RDT Test showed very good sensitivity and specificity, with an excellent agreement with the reference thick blood film microscopy in the identification of both *P. falciparum* and *P. vivax* species. Hence, it is preferable to use the CareStart Malaria *Pf/Pv* Combo Test instead of microscopy for the diagnosis of malaria in areas where microscopy is limited and malaria caused by *P. vivax* and *P. falciparum* is coendemic, like in Ethiopia.

Received October 20, 2013. Accepted for publication February 19, 2014.

Published online March 31, 2014.

Acknowledgments: The authors acknowledge Addis Ababa University for its financial support to conduct this study. Pawe Special Woreda Health Office, Felegeselam Health Center, and Minjar Shenkora Woreda Health Office supported different reagents. Finally, we thank study participants who gave blood samples. The American Society of Tropical Medicine and Hygiene (ASTMH) assisted with publication expenses through the James W. Kazura page waiver fund.

Authors' addresses: Tadesse Hailu, Department of Microbiology, Immunology and Parasitology, College of Medicine and Health Science, Bahir Dar University, Bahir Dar, Ethiopia, E-mail: tadessehailu2005@yahoo.com. Tadesse Kebede, Department of Microbiology, Immunology and Parasitology (DMIP), School of Medicine, Addis Ababa University, Addis Ababa, Ethiopia, E-mail: tkas2003@gmail.com.

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