

ORIGINAL ARTICLE

DIAGNOSTIC PERFORMANCE EVALUATION OF THE SD BIOLINE MALARIA ANTIGEN AG PF/PAN TEST (05FK60) IN A MALARIA ENDEMIC AREA OF SOUTHERN ETHIOPIA

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SUMMARY

Rapid diagnostic tests (RDTs) capable of detecting and differentiating *Plasmodium* species are needed in areas in which microscopy is unsuitable. This study was conducted to assess the diagnostic performance of the rapid test kit - SD BIOLINE Malaria Ag Pf/Pan® (05FK60) in an endemic area. Microscopy of Giemsa-stained blood films were performed to detect and estimate the *Plasmodium* density in malaria suspected patients. The performance of the SD BIOLINE Malaria Ag Pf/Pan test was evaluated using 272 *Plasmodium*-positive and 102 negative blood samples. The overall sensitivity of the SD BIOLINE Malaria Ag Pf/Pan test was 99.5% for *P. falciparum* and 92.6% for non-*P. falciparum* malaria infections. The respective specificity, PPV, and NPV of the test were 98.0, 98.4, and 99.0% for the diagnosis of *P. falciparum*, and 100.0 %, 100.0%, and 94.4% for non-*P. falciparum* species. The SD BIOLINE Malaria Ag Pf/Pan test showed an excellent performance in diagnosing *Plasmodium* infections in an endemic setting. Therefore, this point-of-care test could be used as an alternative to microscopy in places where *P. falciparum* is endemic and microscopy is unsuitable.

**KEYWORDS:** *Plasmodium*; BIOLINE Malaria Ag Pf/Pan; RDT.

INTRODUCTION

Malaria remains a major cause of human morbidity and mortality in many countries of the world. The disease represents a complex public health problem in Africa where 88% and 90% of global cases and deaths occur, respectively<sup>1</sup>. About 75% of the Ethiopian land is potentially taken by malaria, placing over 68% of the total population at risk for this infection<sup>2</sup>. During high malaria transmission seasons, *Plasmodium falciparum* and *P. vivax* account for 60% and 40% of infections, respectively<sup>3</sup>. Vector control using insecticide-treated mosquito nets and indoor residual spraying, as well as case-management through early detection and prompt treatment are among the strategies that the country has implemented to prevent the transmission of malaria<sup>4</sup>.

However, due to a limited access to laboratory services, treatment of malaria is mostly prescribed on the basis of a non-specific clinical diagnosis, which leads to delay and mismanagement. Thus, the need of laboratory confirmation has been emphasized to enhance rapid cure and prevent the emergence of drug resistance<sup>1</sup>. Moreover, the accurate diagnosis of malaria improves the cost-effectiveness of using the newly introduced artemisinin combination therapies<sup>5</sup>. Laboratory services should, therefore, be scaled up and available to meet the increased demand of cases confirmation at peripheral health institutions in which

most malaria patients are treated.

The microscopic examination of stained blood smears remains the gold standard for the diagnosis of malaria. The method has good sensitivity; allows species and stage identification and also the determination of parasitaemia<sup>6</sup>. Despite these strengths, microscopy is time consuming, labor-intensive and often requires access to electricity and skilled personnel<sup>7</sup>, limiting its diagnostic suitability at peripheral health care settings with resource constraints. Thus, alternative malaria diagnostic methods that can be easily established and sustained in poor settings are needed.

Rapid diagnostic tests (RDTs) have been developed to diagnose malaria in places in which reliable microscopy may not be available. These tests commonly target malaria antigens: Histidine-Rich Protein-2 (HRP-2), parasite-specific lactate dehydrogenase (pLDH) and aldolase enzymes. HRP-2 is produced only by *P. falciparum* and persists in the circulation for several weeks after successful treatments. In contrast, parasite LDH and aldolase enzymes are produced by all four *Plasmodium* species (*P. falciparum*, *vivax*, *malariae*, *ovale*) and become undetectable after the initiation of effective therapies. However, the detection of any of these antigens is not useful in monitoring the response to treatment as both sexual and asexual stages of *Plasmodium* express them<sup>8</sup>.

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RDTs are simple to perform and can be handled by a variety of healthcare workers, increasing the number of people that can be tested for malaria. However, the performance of these tests needs to be extensively evaluated to ensure that they have acceptable diagnostic value as heterogeneous results have been documented in different settings. The diagnostic performances of various RDTs have been validated in Ethiopia, in different times. These commercial kits have shown good results in places where microscopy is unavailable. The SD BIOLINE Malaria antigen kit is capable of detecting and differentiating malaria species and has been widely used, replacing the Paracheck Pf and the CareStart test kits. However, the performance of the SD BIOLINE Malaria Ag Pf/Pan (05FK60) test needs to be assessed in several localities so as to determine its diagnostic usefulness. Therefore, this study was conducted to assess the SD BIOLINE Malaria Ag Pf/Pan kit performance in comparison with the microscopic examination of Giemsa-stained blood films in an area in which both *P. falciparum* and *P. vivax* are endemic.

## MATERIALS AND METHODS

### Patients

The study was conducted in the *Arbamich Zuria* District of the *Gamogoffa* zone, Southern Ethiopia. The selection of the study district was based on documented data showing that the district is endemic for malaria. The incidence of malaria increases in the area after the end of the rainy season, and this study was conducted from December, 2014 to February, 2015, a time when malaria transmission is presumed to reach its peak.

Consecutive 374 blood samples (272 positive and 102 negative for malaria) were collected from malaria suspected patients. Individuals who received anti-malaria treatment a month prior to the study recruitment were excluded.

### Blood collection and testing

Study participants were interviewed on socio-demographic characteristics using structured questionnaires. Two milliliters of venous blood were collected from each participant, transferred to EDTA tubes and used to the malaria rapid test and the microscopy. At the same time, additional finger-prick blood samples were to be used in the SD BIOLINE Malaria Ag Pf/Pan® (05FK60) test (Standard Diagnostics Inc., Suwon City, South Korea) following the manufacturer's instructions.

### Microscopy

Thick and thin blood films were prepared, stained with Giemsa and examined to identify and quantify *Plasmodium* species. A minimum of 200 consecutive microscopic fields in a thick blood film were scanned before a negative result was delivered. Parasites were counted considering 200 to 500 white blood cells in a thick blood film, and the parasite density was estimated assuming the total white blood cell count as 8,000 cells/ $\mu$ L of blood.

### SD BIOLINE Malaria Ag Pf/Pan® (05FK60)

The SD BIOLINE Malaria Ag Pf/Pan test contains a cassette format membrane strip and a differential test for the detection of the HRP-2

antigen that is specific to *P. falciparum* and the pLDH antigen that is specific to the *Plasmodium* species. The membrane strip is pre-coated with monoclonal antibodies as three separate lines: a control line which indicates whether the test is valid or not (line 1), a single pan line indicating infections due to *Plasmodium* species (line 2), and a Pf line indicating infections due to the *P. falciparum* species (line 3). The visualization of both lines, i.e., a Pf line and a pan line indicates either a mixed infection with *P. falciparum* and another *Plasmodium* species or a *P. falciparum* infection only. The test procedure is to add 5  $\mu$ L of blood and two drops (60  $\mu$ L) of buffer into the sample well and the diluent well, respectively. The result is read after 20 min. In case the control line did not appear, the result was interpreted as invalid and the test was repeated.

### Quality control

All the test procedures and the interpretation of results were accomplished following the manufacturer's instructions. One experienced microscopist working at the Health Center analyzed all the blood films and determined the parasite density. Another laboratory technician from the same Health Center, who was blinded with respect to the microscopy results, carried out the RDT. The principal investigator has analyzed all the discordant results, and checked 20% of positive and 10% of negative slides.

### Data analysis

Data was analyzed using the SPSS software, version 12.0, and results were summarized using mean, median, range and proportion, as appropriate. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated using microscopy as the gold standard.

### Ethical considerations

The study was approved by the Institutional Review Board of the College of Medicine and Health Sciences, *Hawassa* University. Participation was fully voluntary and written consent was obtained from each study participant or their parents. All the diagnosed malaria cases were treated according to the national guidelines for malaria treatment<sup>3</sup>.

## RESULTS

### Study subjects

A total of 374 blood samples collected from 203 men and 171 women with signs/symptoms suggestive of malaria were investigated in this study. The median age of the study participants was 20 years (range 1 to 80 years). Twenty one participants (5.6%) were under 5 years of age; 83 (22.2%) were between 5 to 15 years, and 270 (72.2 %) were above 15 years old.

### Microscopy

From the total of 374 samples, 81 were positive for *P. vivax*; 187 were positive for *P. falciparum*, and 4 samples had mixed infections of both species. The remaining 102 blood samples were negative for *Plasmodium* species by microscopy (thick blood smear).

Among the infected patients, excluding the mixed infections, 42.5%

(114/268) had parasite densities ranging between 1 and 500 parasites/ $\mu$ L. A density greater than 500 parasites/ $\mu$ L was observed in 70.0% (131/187) of patients with *P. falciparum* and in 28.4% (23/81) of patients with *P. vivax* infections.

### Rapid diagnostic tests

The results of the SD BIOLINE Malaria Ag Pf/Pan test compared with the microscopy are shown in Table 1. The kit detected HRP-2 antigens in 193 patients; of which, 190 samples had also positive results by microscopy. Moreover, 180 negative results were concordant with the microscopy, yielding an overall RDT's accuracy of 98.9% for the detection of *P. falciparum* infection (excluding mixed infections). The SD BIOLINE Malaria Ag Pf/Pan test produced 79 and 289 concordant positive and negative results, respectively, with respect to the microscopy, for non-*P. falciparum* infections. However, the test produced false negative results in one and six samples containing *P. falciparum* and non-*P. falciparum*, respectively.

The sensitivity, specificity, PPV and NPV of the SD BIOLINE Malaria Ag Pf/pan test for the detection of *P. falciparum* infection was 99.5, 98.0, 98.4 and 99.0%, respectively. The sensitivity and specificity of the test for the detection of non-*P. falciparum* infection was 92.6 and 100.0%, respectively, with PPV 100.0% and NPV 94.4 % (Table 2).

The performance of the SD BIOLINE Malaria Ag Pf/pan test was shown to improve with increasing parasite densities. In cases of *P. falciparum* infections, the sensitivity of the test was 96.3% and 100.0% at parasitaemia levels < 100 parasites/ $\mu$ L and > 1,000 parasites/ $\mu$ L, respectively. For non-*falciparum* malaria, the kit showed a sensitivity of 89.2% and 100.0% for blood samples with parasitaemia 1-100 parasites/ $\mu$ L and > 1,000 parasites/ $\mu$ L, respectively. The diagnostic sensitivity of the

test was lower for the detection of non-*falciparum* malaria and in these cases the maximum sensitivity was 95.4%, observed in blood samples with parasitaemia  $\geq$  100 parasites/ $\mu$ L (Table 3).

### DISCUSSION

In this study, the performance of the SD BIOLINE Malaria Ag Pf/ Pan<sup>®</sup> (05FK60) test was evaluated and compared with the microscopy for the diagnosis of malaria in an endemic area. The test is a three-band malaria rapid diagnostic test detecting *P. falciparum* (HRP-2) and *Plasmodium* species (pLDH). The study showed high sensitivity (99.5%) and specificity (98.0%) of the SD BIOLINE Malaria Ag Pf/Pan for detection of *P. falciparum* infection. Moreover, the test has excellent sensitivity to detect non-*P. falciparum* infections.

The sensitivity reported in this study for the SD BIOLINE Malaria Ag Pf/Pan was excellent compared with the previous malaria rapid diagnostic test kits<sup>9,10</sup>. Besides, this kit fulfills the performance requirement recommended for a malaria RDT<sup>11,12</sup>, i.e., a sensitivity greater than 95.0% for samples with parasitaemia  $\geq$  100 parasites/ $\mu$ L of blood. The observed specificity of the SD BIOLINE Malaria Ag Pf/Pan malaria test to detect samples with no *P. falciparum* infections was also in agreement with a similar study conducted in an endemic area of Thailand (99.0%)<sup>13</sup>. However, other similar studies conducted in Central African Republic<sup>14</sup> and Madagascar<sup>15</sup> reported a lower sensitivity, of 88.2% and 92.2%, respectively. The sensitivity differences may be due to various factors that influence the performance of RDTs, including the level of parasitaemia and the genetic diversity of *Plasmodium* species that can vary according to the level of endemicity and the geographical area<sup>16</sup>.

Consistent with previous results<sup>13,10</sup>, the test produced a sensitivity

**Table 1**

Results of the diagnosis of *Plasmodium* species using the SD BIOLINE Malaria Ag Pf/pan (05FK60) test in comparison with the microscopic examination, Southern Ethiopia, 2014-2015

Microscopy result	N°. of samples	SD BIOLINE Malaria Ag Pf/pan (05FK60)			
		Control band only	Pf band only	Pan band only	Pf and pan band
No parasites seen	102	100	2	0	0
<i>P. falciparum</i> only	187	1	66	0	120
<i>P. vivax</i> only	81	6	0	74	1
Mixed Pf and Pv	4	0	0	0	4
<b>Total</b>	<b>374</b>	<b>170</b>	<b>68</b>	<b>74</b>	<b>125</b>

Pf: *P. falciparum*, Pv: *P. vivax*

**Table 2**

Diagnostic performance of the SD BIOLINE Malaria Ag Pf/pan (05FK60) compared with the microscopic examination (excluding mixed infections), Southern Ethiopia, 2014-2015

<i>Plasmodium</i> species	% Sensitivity (95 % CI)	% Specificity (95 % CI)	% PPV (95 % CI)	NPV (95 % CI)
<i>P. falciparum</i>	99.5 (97.1-100.0)	98.0 (93.1-99.8)	98.4 (94.6-100.0)	99.0 (94.6-100.0)
non- <i>P. falciparum</i> species	92.6 (84.6-97.3)	100.0 (96.4-100.0)	100.0 (95.2-100.0)	94.4 (88.3-97.9)

PPV: positive predictive value; NPV: negative predictive value

**Table 3**

Sensitivity of the SD BIOLINE Malaria Ag Pf/pan (05FK60) test at various parasite densities (excluding mixed infections), Southern Ethiopia, 2014-2015

Parasite density	SD 05FK60 test result					
	<i>P. falciparum</i>			<i>P. vivax</i>		
	Positive	Negative	% Sensitivity	Positive	Negative	% Sensitivity
1-100	26	1	96.3	33	4	89.2
101 -500	29	0	100.0	20	1	95.2
501 – 1,000	30	0	100.0	21	1	95.4
> 1,000	101	0	100.0	1	0	100.0
Total	186	1	99.5	75	6	92.6

of 92.6% for the detection of *non-P. falciparum* infections. However, considering parasitaemias > 100 parasites/ $\mu$ L, the test produced an acceptable sensitivity which was greater than 95.0% for *non-P. falciparum*, as recommended by WHO<sup>12</sup>.

In comparison with the microscopy, the SD BIOLINE Malaria Ag Pf/Pan test missed to detect one *P. falciparum* and four *P. vivax* infections in individuals presenting parasite densities < 100 parasites/ $\mu$ L of blood. This indicates that the test kit missed samples with lower levels of parasitaemia, and a possible occurrence of lower antigen concentrations in those samples.

The specificity of the BIOLINE Malaria Ag Pf/Pan test found in the present study for the diagnosis of *P. falciparum* (98.0%) and *non-P. falciparum* (100.0%) infections was comparable to the ones of previous studies conducted in Ethiopia<sup>17,18</sup> and in Madagascar<sup>15</sup>; but differs from the lower specificity reported in Central African Republic<sup>14</sup>. The two false positive results for *P. falciparum* infection in the current study may be due to the persistence of HRP2 in resolved infections<sup>19,20</sup>. In addition, the parasite sequestration, a phenomenon that limits the number of circulating parasites and affects the result of the microscopy may not have a similar influence on the BIOLINE Malaria Ag Pf/Pan test regarding antigenic detection<sup>20, 21, 22</sup>.

The high sensitivity and specificity shown in this study indicate the suitability of the test to perform accurate and timely diagnosis of *P. falciparum* infections, as well as *non-P. falciparum* ones. Moreover, the individual-use disposable blood transfer devices (inverted cup) that are provided with the test kit ensure accuracy and consistency of blood volume transfer to the well on the RDT cassette and make the procedure safer and user-friendly. Therefore, the test kit can be used as an alternative to the microscopy in our settings and will help to avoid delay of diagnosis and mistreatment, which ultimately leads to higher mortality rates and the emergence drug resistance.

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#### COMPETING INTERESTS

The authors would like to declare that no potential competing interests exist.

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