

Effectiveness of RDT and Microscopy in Detection of Malaria Parasite

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Abstract

Malaria presents a diagnostic challenge to laboratories especially in non-endemic malaria countries. Population movements and Travellers all contribute to presenting the laboratory with diagnostic problems for which it may have little expertise available. This study was aimed to compare the accuracy of two malaria Rapid Diagnostic Tests (RDTs); Optimal-IT® and one step RDT with the microscopic examination as the gold standard. Between June 2013 to January 2015 blood samples were collected from 78 patients, attending the internal medicine department at King Faisal specialized hospital at Taif, Saudi Arabia. Microscopy and Rapid Diagnostic Tests (RDTs) were done locally and the accuracy was evaluated. In this study detected 61 (78%) malaria positive slides, while 65 (83%) and 64 (82%) positive samples were detected with Optimal-IT and One Step Malaria Antigen Rapid Tests respectively. Optimal-IT sensitivity (98%), specificity (71%), positive predictive value (92%), negative predictive value (92%) and diagnostic accuracy (92%) whereas, one step RDT test showed 97%, 71 %, 92%, 86%, 91% respectively. However, the differences in detection rates of these diagnostic tests were not statistically significant ($P>0.05$). RDTs are considered the best option in health areas specially with limited laboratory services despite the problems related to their accuracy. However, this study showed that the accuracy of RDTs is not always satisfactory when performed in clinical laboratories.

Keywords: Rapid Diagnostic Test; Malaria; Optimal-IT®; One Step Malaria Antigen Rapid Test

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Introduction

Malaria is one of the most widespread life-threatening infection and one of the major cause of death in many countries, globally killing over one million people annually with 90% of fatalities occur in African children [1, 2] According to the World Health Organization (WHO), most of the infected cases were among children under the age of 5 years [3]. Moreover, in Europe and Mediterranean countries there were an increasing at number of malaria cases during last few years, probably as a result of increasing prevalence of drug-resistant strains of parasites, increasing number of international travel and immigration from malaria endemic countries [2].

Effective control and management of malaria require quick, sensitive, accurate and cost-effective diagnostic methods. Despite the presence of the traditional microscopic examination of stained thin and thick blood films, which currently considered the “gold standard” for malaria diagnosis, this method recognized as difficult and remains a problematic? Such procedure is time-consuming, requires considerable training, an expertise in microscopic examination, well-maintained microscope, and well-stained slides [4]. In addition, errors with microscopy diagnostic were commonly reported for low-density parasitaemia (10 to 100 parasites/ μ l of blood), but errors of quantification also occurred with densities of $>5000/\mu$ l and especially $>20000/\mu$ l of blood [5]. Thus, new effective and sensitive technologies have been developed and introduced to overcome these limitations.

In the last few Years Rapid Diagnostic Tests (RDTs) have been recognized as an ideal method for diagnosing several infectious diseases, including malaria [6]. One of these RDTs for malaria investigation is Opti-MAL. Currently, more than eighty malaria RDTs are available from 28 different manufacturers [3, 7]. Malaria Rapid Diagnostic Tests (RDTs) don't require laboratory equipments and able to detect malaria antigen in blood flowing along a membrane containing specific anti-malaria antibodies. In addition, RDTs can bring significant advantages in malaria control and management when a clear plan of action has been prepared to deal with the outcomes (i.e. drug treatment or appropriate further investigation) [3]. The majority of RDTs are able to detect a *P. falciparum*-specific protein, e.g. Histidine-Rich Protein II (HRP-II) or Lactate Dehydrogenase (LDH), *P. falciparum* specific and pan-specific antigens (aldolase or pan-malaria pLDH), and distinguish non-*P. Falciparum* infections from mixed malaria infections [8, 9].

The large majority of published research studies have evaluated that RDTs have an excellent and accurate performance for diagnosis of malaria [4, 7, 10, 11]. However, wide variations in the sensitivity of RDTs were reported [6, 12]. Additionally, false-negative results might be generated by using malaria RDTs in testing samples with low levels of parasitaemia [6]. Thus, WHO recommended parasitologist who are using RDTs for malaria diagnosis, to be used in conjunction with other methods to confirm the results, characterize infection, and monitor treatment [1].

The aim of this study was to assess and compare between the accuracy of the LDH-based RDT (Optimal) and One Step Malaria Antigen Rapid Test Bio Line with the traditional microscopic examination of stained thin and thick blood films, which is "gold standard" for malaria diagnosis in King Faisal specialized hospital at Taif, Saudi Arabia.

Subjects and Methods

This descriptive study was carried on 78 patients presenting with fever $>38^{\circ}\text{C}$ and were clinically diagnosed as malaria infection. The patients were 73 (94%) males and 5 (6%) females with an average age of 14-57 years, attending the internal medicine department at King Faisal specialized hospital, Taif,

Saudi Arabia, in the period from June 2013 to January 2015. Most patients were workers from outside KSA. Blood obtained by vein puncture collected on EDTA tubes when patients required to be tested for malaria. Shortly after being drawn to prevent alteration in the morphology of blood cells and/or malaria parasites both thick and thin blood films were prepared and examined for the presence of *Plasmodium* parasites, also OptiMAL test and one step RDT were done.

Microscopic Examination of Blood Smears

Thick and thin blood films were prepared, stained with fresh 10% Giemsa's solution and examined using X 1000 oil immersion magnification. The slides were reported negative only when no parasites were detected in 200 fields of each thick film. Stained thin film preparations of positive thick films were examined to determine the species: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* or mixed infection. Parasitaemia was evaluated in 100 fields of thin films against the leucocytes counts taken from records of the patients to check the density of infection, based on the equation: $\text{number of parasites}/\mu\text{l} = \text{total parasite count}/\text{WBC count} \times \text{the total leucocyte count}/\mu\text{l}$

Opti-MAL Test

Optimal-IT® malaria test (Diamed, Flow Inc. Portland, Oreg.) was performed according to the manufacturer's instructions. Briefly, a drop of blood was added to a well in a micro titer plate and mixed with two drops of lysis buffer A, which disrupts the red blood cells and releases the pLDH. The specimens were then allowed to migrate to the top of the pLDH strip. After eight minutes, the strips were placed in washing buffer B, which clears the haemoglobin from the strip. Positive and negative control samples were included with each batch tested. The entire process took approximately 15 min, and results were visually interpreted immediately. A positive control line was always presented at the top of the strip to verify that the test strip was functional. If this was the only line that appeared, the test was considered negative for malaria. Appearance of a second line, adjacent to the positive control line, indicated the presence of a non-*P. falciparum* malaria parasite (*P. vivax*, *P. ovalae*, or *P. malariae*). When a third line was also presented, this indicated a positive response for *P. falciparum* infection.

One Step Malaria Antigen Rapid Test Bio Line (STANDARD DIAGNOSTICS INC., Germany)

This rapid test was performed according to the manufacturer' instructions. Briefly, all kit components and specimen were kept at 10 minutes at room temperature prior to test. Test device was removed from foil pouch and placed on a flat, dry surface. Patient's fingertip was cleaned and pricked with lancet and 5µl capillary pipette provided; whole blood specimen was drawn to black line and then transferred into the round sample well. Then, 4 drops of assay diluent were added into the square assay diluent well. After 15 minutes (up to 30 minutes) result was read. Reading of the result was avoided after 30 minutes because reading too late can give false results. A negative result was indicated at the presence of one color band ("C" Control line) within the result window. While the presence of two color bands ("P.f" Test line and "C" Control line) within the result window, no matter which band appeared first, indicated P.f positive result. The presence of two color bands ("Pan" Test line and "C" Control line) within the result window indicated Pan (P.v or P.m or P.o) positive result. The presence of three color bands ("P.f", "Pan" Test lines and "C" Control line) within the result window, indicated P.f positive or mixed infection of P.f and P.v or P.m or P.o. If the control band ("C" Control line) failed to appear within the result window, the result was considered invalid and the specimen was retested.

Statistical Analysis

Data were collected and analyzed statistically using SPSS version 19. The numbers of True Positives (TP), True Negatives (TN), False Positives (FP) and False Negatives (FN). were used to calculate: Sensitivity was $TP / (TP + FN) \times 100$, specificity as $TN / (TN + FP) \times 100$, the positive predictive value (PPV) as $TP / (TP + FP) \times 100$, and the negative predictive value (NPV) as $TN / (FN + TN) \times 100$, Diagnostic Accuracy (DA) as $TP + TN / \text{Total No. of patients} \times 100$.

Ethical Approval

Ethical approval for this study was provided by Ethical committees of the King Faisal specialized hospital.

Results

From June 2013 to January 2015, 78 patients were screened at the internal medicine department at King Faisal specialized hospital for the presence of malaria. Patients were aged between 14 to 57 years, 93% were male and 7% were female, 87% were non-Saudi, while Saudi patients were only 13% (Figure 1). About 61 (78%) malaria positive slides were detected of whom 21 (36%) were *P. falciparum*, 39 (64%) were *P. vivax* (Table 2). Optimal-IT gave positive results in 65 (83%) and One Step Malaria Antigen Rapid Test Bio Line in 64 (82%) patients (Table 1).

Figure1: Number of patients belongs to different nationality involved in the study.

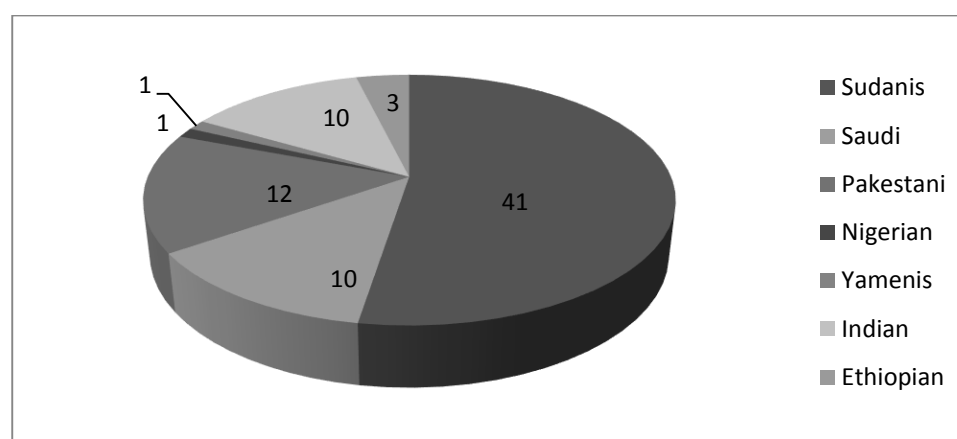


Table 1: Overall sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) and Diagnostic Accuracy (DA) of malaria rapid diagnostic methods with expert microscopy as gold standard. OptiMAL test vs direct microscopy, $P > 0.05$ = no significant difference. One step test vs direct microscopy, $P > 0.05$ = no significant difference. OptiMAL test vs One step test, $P > 0.05$ = no significant difference.

Applied tests	Direct microscopy			Sens.	Spec.	PPV	NPV	DA
	+ve (%)	-ve (%)	Total (%)	%	%	%	%	%
OptiMAL								
Positive	60 (77%)	5 (7%)	65 (83%)	98	71	92	92	92
Negative	1 (1%)	12 (15%)	13 (17%)					
Total	61 (78%)	17 (22%)	78 (100%)					
One step								
Positive	59 (76%)	5 (7%)	64 (82%)	97	71	92	86	91
Negative	2 (2%)	12 (15%)	14 (18%)					
Total	61 (78%)	17 (22%)	78 (100%)					

Table 2: Results of microscopy for detection of malaria species

Direct microscopy	Malaria species				Total
	<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. malariae</i>	<i>P. ovale</i>	
Positive	39 (64%)	21 (36%)	0 (0%)	0 (0%)	61 (77%)
Negative					17 (23%)
Total					78 (100%)

Considering the expert microscopy as the gold standard, method for malaria detection, the sensitivity of the RDTs, which used in this study was 98% and 97% for Optimal-IT and One Step Malaria Antigen Rapid Test Bio Line respectively. However, the specificity was exactly similar for both RDTs with 71% (Table 1).

From the results of this study there was no technique reached the Positive Predictive Value (PPV) of 95%, whereas the Negative Predictive Value (NPV) was over 90% for Optimal-IT and 86% for One Step Malaria Antigen Rapid Test (Table 1).

Discussion

In 2014, WHO reported that about 3.3 billion people (almost half of the world's population) were prone to the risk of malaria. As a result, about 216 million people are affected by the disease each year [13].

Our results showed that most cases affected with malaria were non –Saudi (Indian, Sudanese, Pakistani, Ethiopian, Nigerian and Yemeni) as shown in figure 1, this result is also matching with previous study done by Abd-Elwahab et al., [14] who reported increase malarial cases in immigrant workers in

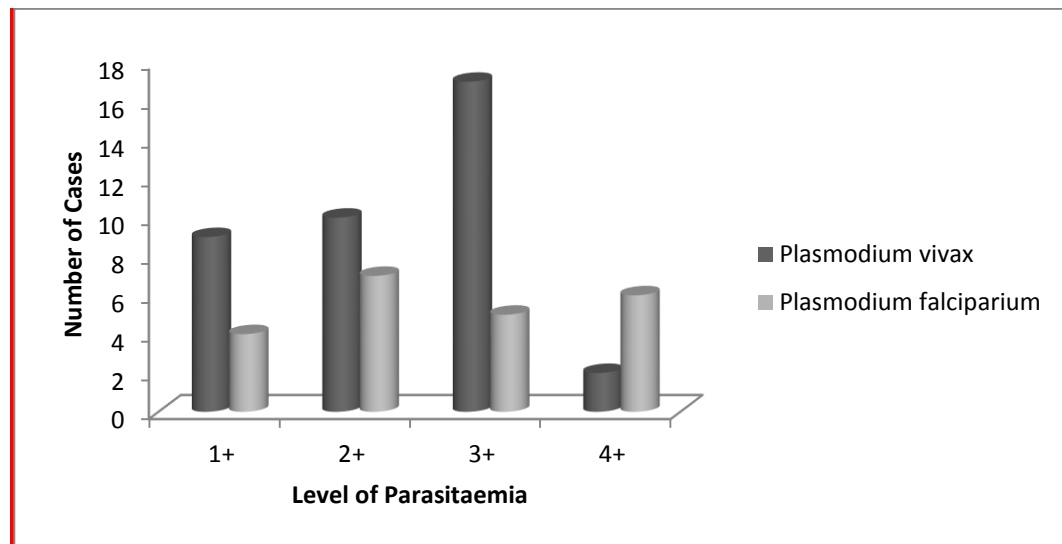
Saudi. Moreover, in this study most of our cases were *P.vivax* infected patients 64% followed by *P.falciparum* 36% as shown in table 2 as most of our cases were from southeast of Asia and the dominant species were *P.vivax*, which is similar to the results shown by Ngasala et al., [15]. The level of parasitaemia was higher in *P.vivax* cases as shown in figure 2, this result is matching with the finding of Jang et al., [16] as most of their cases were with high *P.vivax* Parasitaemia.

A number of published studies were performed to assessing the sensitivity and specificity of different malaria rapid diagnostic tests such as Optimal-IT, BinaxNOW and Paracheck-PfW [4, 7, 10, 11]. In this study, the sensitivity of Optimal-IT reached the acceptable threshold of 90% as shown in table 1, while it showed only 77% of sensitivity in other study done in 2012 Muhindo et al., [4]. This variation in test sensitivity could be generated as a result of environmental effect like moisture temperature, or reading the result following different time, also level of Parasitaemia may be another factor.

Figure 2: Level of *Parasitaemia* in different plasmodium species

By thick blood film

- 1+ 1-10 parasites per 100 HPF
- 2+ 11-100 parasites per 100 HPF
- 3+ 1-10 parasites per each HPF
- 4+ > 10 parasites per each HPF



The sensitivity and specificity of the one step RDT were 97% and 71%, respectively, compared with light microscopy (Table 1). The corresponding positive, negative predictive values and diagnostic accuracy were 92%, 86% and 91% respectively in contrast to result obtained by Ngasala et al., [15] this may be due to the difference of the species of malaria.

In explanation of false positivity researchers hypothesized that RDT positive cases missed by microscopy might be individuals who had been treated but in whom antigenemia persists [17]. Other reasons include persistence of antigens due to sequestration of malaria parasites from peripheral blood [18], incomplete treatment, delayed clearance of circulating antigen (free or in anti- gen-antibody complexes), and cross reaction with nonfalciparum malaria, rheumatoid factor [19], or heterophile antibodies [12]. False negative results of RDTs have been attributed to possible genetic heterogeneity of HRP2 or LDH

expression, deletion or mutation of HRP2 or LDH gene, presence of blocking antibodies, or immune complex formation[19], and also inability of OptiMAL test to detect parasitaemia levels blow 100 parasites/ μ l of blood [20] (Figure 3). Since, malaria is one of the most widespread life-threatening infection, misdiagnosis of malaria may result in significant morbidity and mortality. Thus, rapid, sensitive and accurate detection of malaria parasites has an important role in promoting rapid diagnosis and treatment. Moreover, RDTs offer the potential to provide accurate diagnosis to all at- risk populations for the first time, reaching those unable to access good quality microscopy services. Despite the limitation and problems related to accuracy of malaria RDTs, they are considered the best option in many laboratories with limited services. However, parasitologists who are using RDTs for malaria diagnosis were required to use other methods to confirm the results and monitor treatment [1] (Figure 4).

Figure 3: Resulting reaction on the OptiMAL test strip

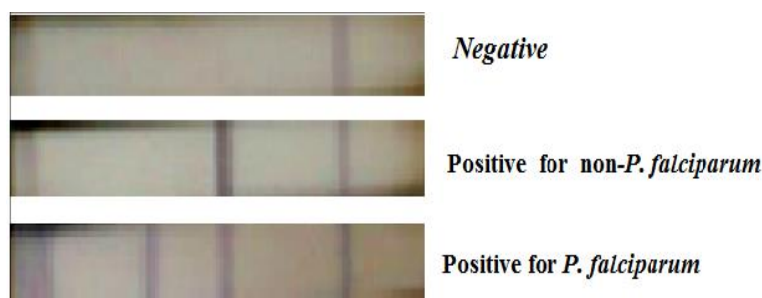
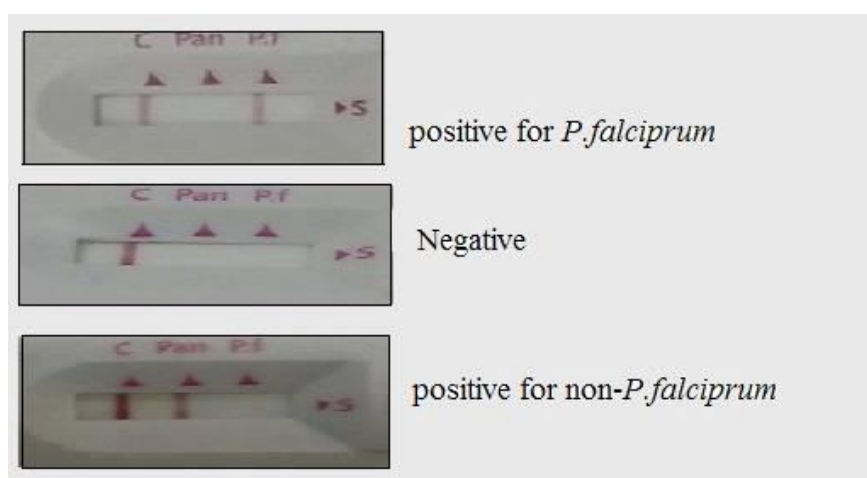


Figure 4: Resulting reaction on the One step test strip



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