EVALUATION OF MALARIA RAPID DIAGNOSTIC TESTS IN MADINAH, SAUDI ARABIA

Majed H Wakid[1,2] and Ziab Z Alahmadey[3]

[1]Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, [2]Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah; [3]Ministry of Health, Al-Ansar Hospital, Madinah, Saudi Arabia

Abstract. Malaria is a parasitic disease causing high morbidity and mortality in tropical and sub-tropical regions of the world. Determination of malaria parasitemia level is essential for estimating severity of the disease. Detection of malaria in endemic regions using rapid diagnostic tests (RDTs) has been widely adopted. The study evaluated prevalence of *Plasmodium* spp. in Madinah, Saudi Arabia, determined parasite density and assessed diagnostic accuracy of RDTs. One hundred EDTA blood samples were collected from patients presenting fever or a recent history of fever and examined microscopically in parallel with two RDTs (OptiMAL-IT and AMP). Malaria was microscopically confirmed in 20% of the samples, with *Plasmodium falciparum* and *P. vivax* detected in 13 and 7% respectively and no mixed infection. Mean parasite density for *P. falciparum* and *P. vivax* was 6,357 and 5,660 parasites/μl respectively. Sensitivity of AMP and OptiMAL-IT tests was 85 and 80% respectively, and 100% specificity for both tests. In conclusion, diagnostic performance of the two RDTs were satisfactory with AMP having a slightly higher sensitivity than OptiMAL-IT test, but both RTDs were still inferior compared to microscopic examination.

Keywords: *Plasmodium falciparum*, *Plasmodium vivax*, AMP test, OptiMAL-IT test, parasitemia

INTRODUCTION

Malaria still remains a major life-threatening disease and is caused by *Plasmodium* protozoan parasite transmitted from bites of infected *Anopheles* mosquitoes (WHO, 2018a). In 2017, World Health Organization (WHO) estimated globally there were 219 million new cases of malaria with a mortality of 435,000 (WHO, 2018b). It is advisable to have rapid and accurate malaria diagnosis before commencing treatment to minimize morbidity and mortality (WHO, 2010; FIND, 2019; WHO, 2018a).

The common routine diagnosis is carried out by microscopy and by rapid diagnostic tests (RDTs). Light microscopy examination using thin and thick blood films is considered as the "gold standard" (Salimi Khorashad *et al*, 2014); however, the method is labor intensive, requires skilled technicians and (of course) needs a light microscope. Several commercial

Correspondence: Majed H Wakid, Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, PO Box 80324, Jeddah 21589, Saudi Arabia.
Tel: +966 503627311
Fax: +966 26952000 ext. 25502
E-mail: mwakid@kau.edu.sa

RDTs are available to detect specific targets of *Plasmodium* parasites, based on immunochromatographic assay on a nitrocellulose strip (Gillet *et al*, 2010; WHO, 2010). The test targets either *Plasmodium* sp-specific histidine rich protein-2 (HRP2) or pan-*Plasmodium*-specific target dehydrogenase (pan-pLDH) or aldolase (Gillet *et al*, 2010). RDTs are simple and easy to carry out and do not require laboratory instruments or special tools (Gillet *et al*, 2010; WHO, 2018b). Output is in the form of line(s) appearing on the strip within a few minutes, which can be directly interpreted as a positive or negative result (Gitonga *et al*, 2012).

The study was conducted to evaluate the diagnostic accuracy, reproducibility, and efficiency of two RDTs (AMP and OptiMAL-IT) in comparison with the gold standard microscopy among blood samples from patients attending the General Hospital, Madinah, Saudi Arabia.

MATERIALS AND METHODS

Specimens collection

Patients (n = 100) attending the General Hospital, Madinah, Saudi Arabia, with clinical symptoms and signs of suspected malaria infection were recruited. EDTA blood samples were collected and sent immediately to the parasitology laboratory. Demographic information was retrieved from medical records.

The research protocol study was approved by the Ministry of Health, Directorate of Health Affairs, Madinah, Saudi Arabia (approval no. IRB 330). No prior consent was required as the protocol was part of routine clinical examinations and names of patients were removed prior to dissemination of medical records.

Blood film examination and parasitemia determination

Thick and thin blood films were stained with 10% Giemsa stain and at least 100 microscopic fields examined under a light microscope (100x magnification) (Haggaz *et al*, 2014; Salimi Khorashad *et al*, 2014). In positive cases, parasitemia level was determined using two methods (WHO, 2010; Salimi Khorashad *et al*, 2014): (i) number of parasites/μl in thick blood film quantified using the formula (assuming mean count of white blood cells (WBCs) of 8000/μl blood):

$$\text{Number of parasites}/\mu\text{l blood} = \frac{\text{Number of parasites counted}}{\text{Number of WBCs counted}} \times 8000$$

and (ii) percent infected red blood cells (RBCs) in thin blood film (300-500 cells) calculated using the formula:

$$\text{Percent parasitemia} = \frac{\text{Number of infected RBC}}{\text{Total number of RBCs counted}} \times 100$$

AMP test

AMP test, an immunochromatography RTD (AMEDA cat no. RT2655; AMEDA Labordiagnostik GmbH, Graz, Austria) is based on detection of *P. falciparum*-specific histidine rich protein 2 (HRP2) and non-*falciparum*-specific lactate dehydrogenase (pLDH) in whole blood sample using monoclonal antibodies (Maltha *et al*, 2012). A 5 μl aliquot of EDTA blood was added to well "A" and three drops (approximately 180 μl) of the buffer to buffer well "B". After 10 minutes, the test result is interpreted as positive *P. falciparum*, when two bands develop (control and "Pf" lines); positive pan non-*falciparum* malaria, when two bands develop (control and "P" lines); positive mixed infection, when three bands develop (control, "Pf" and "P" lines); and negative, when only control

line develops a band.

OptiMAL-IT test

OptiMAL-IT test, an immunochromatographic RDT (BIO-RAD cat no. 710024; BIO-RAD, Hercules, CA), is based on detection of *P. falciparum* and pan-specific (non-*falciparum* species) lactate dehydrogenase (pLDH) using monoclonal antibodies (Gitonga *et al*, 2012; Maltha *et al*, 2012). One drop of buffer was added to "conjugate well", four drops were added to "wash well" and 5 µl aliquot of EDTA blood was added to the "conjugate well". After one minute, the one end of a dipstick was inserted in the "conjugate well" and allowed to stand for 10 minutes, then transferred to the "wash well" and allowed to stand for another 10 minutes before observing the result. When bands appear at both control and test "Pf" lines, the result is considered positive for *P. falciparum*; if bands appear at control and test "P" lines, the result is considered positive for non-*falciparum* malaria; if three bands appear, the result is considered positive for mixed infection; and if only a band appears at control line, the result is considered negative.

Statistical analysis

Results were analyzed using the Statistical Package for the Social Sciences (SPSS) version 22 (SPSS Inc, Chicago, IL). A p-value <0.050 is considered statistically significant.

RESULTS

Of the 100 EDTA blood specimens, 20 were malaria-positive by microscopy, with subjects of 18-65 years of age and highest infection rate among patients 41-60 years of age (Table 1). Prevalence of malaria in male and female patients are not statistically significant. Prevalence of infection is significantly higher among non-Saudi: Pakistanis (n = 6), Nigerians

Table 1
Demographic profile of patients attending General Hospital, Madinah, Saudi Arabia.

Characteristic	Number of patients (n = 100)	Positive cases# Number (%)	p-value
Male	58	15 (75)	0.085*
Female	42	5 (25)	
Saudi	17	0 (0)	0.015**
Non-Saudi	83	20 (100)	
<20 years of age	3	1 (5)	0.469*
21-40 years of age	33	3 (15)	
41-60 years of age	58	15 (75)	
>60 years of age	6	1 (5)	
Infected with *P. falciparum*		13 (65)	0.469*
Infected with *P. vivax*		7 (35)	

P. falciparum: *Plasmodium falciparum*; *P. vivax*: *Plasmodium vivax*; #Microscopic examination; *Chi-square test; **Fishers' exact test.

(n = 5), Sudanese (n = 5), Yemenis (n = 3), and Indian (n = 1) compared to Saudi patients. Infection rate is not significantly different between *P. falciparum* and *P. vivax*. Although the range of parasite densities was larger among *P. falciparum* compared to *P. vivax* cases, mean and median values are not significantly different (Table 2).

Compared to the microscopy (gold standard), AMP and OptiMAL-IT test detected 17 and 16% malaria cases, a sensitivity of 85 and 80% respectively, but specificity for both of 100% (Table 3). Positive and negative predictive value for AMP and OptiMAL-IT test was 100 and 96% and 100 and 95% respectively. Sensitivity of AMP and OptiMAL-IT tests was equal that of microscopy at parasite density >5,000 parasites/μl, but fared less well at lower parasite densities (Table 4).

DISCUSSION

Hajj and Umrah religious rituals may introduce malaria into Saudi Arabia, as many Muslim countries have a high prevalence of *P. falciparum* and *P. vivax* infections (Almutairi *et al*, 2018). In addition, hundreds of thousands of foreign migrant workers in Saudi Arabia come from malaria-endemic countries. The results of the study as regards demographic profile of malaria patients were consistent with a recent study by Gomerep *et al* (2017).

The rate of positivity in the present study was similar to previous studies (Houzé *et al*, 2013; Laman *et al*, 2014; Mukry *et al*, 2017). *P. falciparum* and *P. vivax* were the most common plasmodial species in agreement with findings in

Table 2
Plasmodium spp parasitemia from thick blood film examination.

Measurement	Number of parasites/μl	
	Plasmodium falciparum (n = 13)	*Plasmodium vivax* (n = 7)
Minimum	720	3,240
Maximum	12,000	7,360
25th percentile	3,720	5,160
Median	7,520	5,600
75th percentile	8,280	6,920
Mean	6,357	5,660
SD	3,225	1,345
Mean LL (95% CI)	4,408	4,415
Mean UL (95% CI)	8,306	6,904
Range	11,280	4,120
IQR	4,560	1,760

*Based on white blood count of 8,000/μl; μl: microliter; CI: confidence interval; IQR: interquartile range; LL: lower limit; UL, upper limit; SD: standard deviation

Table 3
Comparison of microscopy with malaria rapid diagnostic tests.

Rapid diagnostic test		Microscopy		
		Positive	Negative	Total
AMP test	Positive	17	0	17
	Negative	3	80	83
OptiMAL-IT test	Positive	16	0	16
	Negative	4	80	84
Total		20	80	100

Table 4
Parasite density of malaria positive cases using microscopy and rapid diagnostic tests.

Number of parasites/μl	Number of cases		
	Microscopy	OptiMAL-IT	AMP
<1000	3	0	1
1000-5000	4	3	3
>5000	13	13	13
Total	20	16	17

μl: microliter of blood.

Guinea (Laman *et al*, 2014), Myanmar and Thailand (ACTwatch Group *et al*, 2017) and Vietnam (Thang *et al*, 2009).

Although microscopic examination of thick and/or thin blood film is the gold standard for malaria detection, in order to bypass the inherent weaknesses of the microscopic examination, WHO supports applications of easy, rapid, accurate, inexpensive, and microscope-independent malaria diagnostic tests (WHO, 2010). During 2010-2017, 1.92 billion RDTs were sold worldwide, 66% specific for detection of *P. falciparum* (WHO, 2018b). HRP2-based RDT is the most globally utilized test and has been applied in malaria cases of low and high-density parasitemias (Kyabayinze *et al*, 2011; Aguilar *et al*, 2012). pLDH test appears to perform poorly at low parasite densities (Abba *et al*, 2011). When HRP2-based RDT is used during treatment, microscopy or other tests should be used to confirm the positive results as the antigen persists in the blood even after parasite clearance, unlike pLDH that is secreted only from living parasites (Houzé *et al*, 2009). HRP2 in combination with pLDH test has been used for differentiation between *P. falciparum* and non-*falciparum* infections (Maltha *et al*, 2012).

In the present study sensitivity, specificity, and positive and negative predictive values of AMP and OptiMAL-IT tests were comparable, consistent with previous reports using these RDTs from

Afghanistan (Kolaczinski *et al*, 2004), Ghana (Ayeh-Kumi *et al*, 2011), India (Singh *et al*, 2010), Kuwait (Iqbal *et al*, 2002), Malawi (Makuuchi *et al*, 2017), Saudi Arabia (Alkhiary, 2015), and Turkey (Aslan *et al*, 2001). However, there are reports of HRP2 sensitivity being higher than that of pLDH (Bell and Peeling, 2006; Singh *et al*, 2013). Reduced sensitivity of RDTs at low parasite densities (200-500 parasites/μl) have also been observed (Palmer *et al*, 1998; Gitonga *et al*, 2012), in particular with *P. vivax* infection (McMorrow *et al*, 2011).

Differences between median *P. falciparum* and *P. vivax* densities in thick films are not significant, but other studies showed significant differences (Jeremiah and Uko, 2007; Bilal *et al*, 2016). Reports of lower WBCs in *P. falciparum* compared to *P. vivax* infections are not consistent (McKenzie *et al*, 2005; Tangpukdee *et al*, 2008; Adam *et al*, 2011). Several studies (Adu-Gyasi *et al*, 2012; Alves-Junior *et al*, 2014; Haggaz *et al*, 2014; Liu *et al*, 2016) do not support the use of 8,000 WBCs/μl blood in estimating parasite density (WHO, 2010). It may be necessary to validate this assumption on a situation-by-situation basis.

In conclusion, the study confirms the use of thick and/or thin blood film as the gold standard method in examination of all *Plasmodium* spp infection. Malaria rapid diagnostic tests, AMP and OptiMAL-IT, were only 80% sensitive compared to microscopy but were 100% specific. For accurate high through-put detection of malaria, molecular techniques will be required.

ACKNOWLEDGEMENT

This project was funded by Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant No. 387-142-1440. The authors, therefore, acknowledge with thanks DSR for technical and financial support. Thanks extend to the Ministry of Health, Directorate of Health Affairs in Madinah for support of this study.

REFERENCES

Abba K, Deeks JJ, Olliaro PL, *et al*. Rapid diagnostic tests for diagnosing uncomplicated *P. falciparum* malaria in endemic countries. *Cochrane Database Sys Rev* 2011; 7: CD008122.

ACTwatch Group, Phok S, Phanalasy S, Thein ST, Likhitsup A. Private sector opportunities and threats to achieving malaria elimination in the Greater Mekong Subregion: results from malaria outlet surveys in Cambodia, the Lao PDR, Myanmar, and Thailand. *Malar J* 2017; 16: 180.

Adam I, Elhassan BM, Haggaz AE, Ali AA, Adam GK. A perspective of the epidemiology of malaria and anaemia and their impact on maternal and perinatal outcomes in Sudan. *J Infect Dev Ctries* 2011; 5: 83-7.

Adu-Gyasi D, Adams M, Amoako S, *et al*. Estimating malaria parasite density: assumed white blood cell count of 10,000/μl of blood is appropriate measure in Central Ghana. *Malar J* 2012; 11: 238.

Aguilar R, Machevo S, Menéndez C, *et al*. Comparison of placental blood microscopy and the ICT HRP2 rapid diagnostic test to detect placental malaria. *Trans R Soc Trop Med Hyg* 2012; 106: 573-5.

Alkhiary W. Evaluation of the diagnostic performance of OptiMAL-IT test for the detection of *Plasmodium falciparum* in South-West Saudi Arabia. *J Blood Disord Transfus* 2015; 6: 272.

Almutairi MM, Alsalem WS, Hassanain M, Hotez PJ. Hajj, Umrah, and the neglected tropical diseases. *PLoS Negl Trop Dis* 2018; 12: e0006539.

Alves-Junior ER, Gomes LT, Ribatski-Silva D, *et al*. Assumed white blood cell count of 8,000 cells/μL overestimates malaria parasite density in the Brazilian Amazon. *PLoS One* 2014; 9: e94193.

Aslan G, Ulukanligil M, Seyrek A, Erel O. Diagnostic performance characteristics of rapid dipstick test for *Plasmodium vivax* malaria. *Mem Inst Oswaldo Cruz* 2001; 96: 683-6.

Ayeh-Kumi PF, Akalifa BG, Obeng Nkrumah N, Asmah RH, Dayie NT. Performance of rapid DiaMed OptiMal-IT(®) malaria test in an endemic Ghanaian setting. *J Parasit Dis* 2011; 35: 129-33.

Bell D, Peeling RW. Evaluation of rapid diagnostic tests: malaria. *Nat Rev Microbiol* 2006; 4: S34-8.

Bilal JA, Gasim GI, Karsani AH, Elbashir LM, Adam I. Malaria parasite density estimation using actual and assumed white blood cells count in children in Eastern Sudan. *J Tropical Pediatr* 2016; 62: 171-5.

Foundation for Innovative New Diagnostics (FIND). Malaria rapid diagnostic test: an implementation guide, 2013 [cited 2019 Jul 17]. Available from: URL: https://www.ghdonline.org/uploads/malaria_rdt_implementation_guide2013.pdf

Gillet P, Mukadi P, Vernelen K, *et al*. External quality assessment on the use of malaria rapid diagnostic tests in a non-endemic setting. *Malar J* 2010; 9: 359.

Gitonga CW, Kihara JH, Njenga SM, *et al*. Use of rapid diagnostic tests in malaria school surveys in Kenya: does their under-performance matter for planning malaria control? *Am J Trop Med Hyg* 2012; 87: 1004-11.

Gomerep SS, Terver AM, Oye IH, Ejiji IS, Joseph AO. Prevalence of malaria parasitemia and its association with ABO blood group in Jos, Nigeria. *Int J Infect Dis Ther* 2017; 2: 59-65.

Haggaz AD, Elbashir LM, Adam GK, Rayis DA, Adam I. Estimating malaria parasite density among pregnant women at Central Sudan using actual and assumed white blood cell count. *Malar J* 2014; 13: 6.

Houzé S, Boly MD, Le Bras J, Deloron P, Faucher JF. PfHRP2 and PfLDH antigen detection for monitoring the efficacy of artemisinin-based combination therapy (ACT) in the treatment of uncomplicated *falciparum* malaria. *Malar J* 2009; 8: 211.

Houzé S, Boutron I, Marmorat A, *et al*. Performance of rapid diagnostic tests for imported malaria in clinical practice: results of a national multicenter study. *PLoS One* 2013; 30; 8: e75486.

Iqbal J, Khalid N, Hira PR. Comparison of two commercial assays with expert microscopy for confirmation of symptomatically diagnosed malaria. *J Clin Microbiol* 2002; 40: 4675-8.

Jeremiah ZA, Uko EK. Comparative analysis of malaria parasite density using actual and assumed white blood cell counts. *Ann Trop Paediatr* 2007; 27: 75-9.

Kolaczinski J, Mohammed N, Ali I, *et al*. Comparison of the OptiMAL rapid antigen test with field microscopy for the detection of *Plasmodium vivax* and *P. falciparum*: considerations for the application of the rapid test in Afghanistan. *Ann Trop Med Parasitol* 2004; 98: 15-20.

Kyabayinze DJ, Tibenderana JK, Nassali M, *et al*. Placental *Plasmodium falciparum* malaria infection: operational accuracy of HRP2 rapid diagnostic tests in a malaria endemic setting. *Malar J* 2011; 10: 306.

Laman M, Moore BR, Benjamin J, *et al*. Comparison of an assumed versus measured leukocyte count in parasite density calculations in Papua New Guinean children with uncomplicated malaria. *Malar J* 2014; 13: 145.

Liu H, Feng G, Zeng W, *et al.* A more appropriate white blood cell count for estimating malaria parasite density in *Plasmodium vivax* patients in northeastern Myanmar. *Acta Trop* 2016; 156: 152-6.

Makuuchi R, Jere S, Hasejima N, Chigeda T,

Gausi J. The correlation between malaria RDT (Paracheck pf.®) faint test bands and microscopy in the diagnosis of malaria in Malawi. *BMC Infect Dis* 2017; 17: 317.

Maltha J, Gamboa D, Bebdezu J, *et al*. Rapid diagnostic tests for malaria diagnosis in the Peruvian Amazon: impact of pfhrp2 gene deletions and cross reactions. *PLoS One* 2012; 7: e43094.

McKenzie FE, Prudhomme WA, Magill AJ, *et al*. White blood cell counts and malaria. *J Infect Dis* 2005; 192: 323-30.

McMorrow ML, Aidoo M, Kachur SP. Malaria rapid diagnostic tests in elimination settings can they find the last parasite? *Clin Microbiol Infect* 2011; 17: 1624-31.

Mukry SN, Saud M, Sufaida G, Shaikh K, Naz A, Shamsi TS. Laboratory diagnosis of malaria: comparison of manual and automated diagnostic tests. *Can J Infect Dis Med Microbiol* 2017; 2017: 9286392.

Palmer CJ, Makler M, Klaskala WI, Lindo JF, Baum MK, Ager AL. Increased prevalence of *Plasmodium falciparum* malaria in Honduras, Central America. *Rev Panam Salud Publica* 1998; 4: 40-2.

Salimi Khorashad A, Salehi M, Roshanravan B. The comparison of microscopic method and rapid diagnostic test in detecting *Plasmodium* species. *Int J Infect* 2014; 1: e21441.

Singh N, Bharti PK, Singh MP, *et al*. Comparative evaluation of bivalent malaria rapid diagnostic tests versus traditional methods in field with special reference to heat stability testing in Central India. *Plos One* 2013; 8: e58080.

Singh N, Shukla MM, Shukla MK, *et al*. Field and laboratory comparative evaluation of rapid malaria diagnostic tests versus traditional and molecular techniques in India. *Malar J* 2010; 9: 191.

Tangpukdee N, Yew HS, Krudsood S, *et al*. Dynamic changes in white blood cell counts in uncomplicated *Plasmodium falciparum* and *P. vivax* malaria. *Parasitol Int* 2008; 57: 490-4.

Thang ND, Erhart A, Hung LX, *et al*. Rapid decrease of malaria morbidity following the introduction of community-based monitoring in a rural area of Central Vietnam. *Malar J* 2009; 8: 3.

World Health Organization (WHO). *Basic malaria microscopy*. 2nd ed, 2010 [cited 2019 Jul 17]. Available from: URL: http://whqlibdoc.who.int/publications/2010/9789241547826_eng.pdf

World Health Organization (WHO). Malaria rapid diagnostic test performance. Results of WHO product testing of malaria RDTs: round 8 (2016-2018), 2018a [cited 2019 Jul 17]. Available from: URL: https://www.who.int/malaria/publications/atoz/9789241514965/en/

World Health Organization (WHO). World malaria report 2018, 2018b [cited 2019 Jul 17]. Available from: URL: https://www.who.int/malaria/publications/world-malaria-report-2018/en/