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Validation of Malaria Rapid Diagnostic Tests (RDTS) *vs.* Microscopy

Alshamrani HA[1], Abdel-Wahab MM[2,3], Althobaiti Naif Saad M[4], Alfahmy Sharifah Atiah R[5], Ismail KA[6,7]*, Khalifa AM[8] and Khalifa OM[9]

[1]Chief of Laboratory & Blood Bank at King Faisal Medical Complex, Taif, KSA

[2]Department of Parasitology, Research Institute of Ophthalmology, Giza, Egypt

[3]Department of Medical Parasitology, at King Faisal Medical Complex, Taif, KSA

[4]Laboratory Chief of Technicians at King Faisal Medical Complex, Taif, KSA

[5]Supervisor of TB Unit at King Faisal Medical Complex, Taif, KSA

[6]Department of Applied Medical Science, Taif University, KSA

[7]Department of Medical Parasitology, Ain-Shams University, Cairo, Egypt

[8]Department of Forensic and Toxicology, Ain-Shams University, Cairo, Egypt

[9]Department of Medicine, Ain-Shams University, Cairo, Egypt

Abstract

Saudi Arabia is considered as hypoendemic area for malaria thus its diagnosis is a challenge to many laboratories. For management and control of malaria, prompt and effective diagnostic methods are essential. Traditional methods of malaria diagnosis remain problematic as a result of the little expertise available. Therefore, to get over the limitations, new technologies have been developed and used. This study aimed to compare the accuracy of malaria Rapid Diagnostic Tests (RDTs), CareStart™ and Optimal Malaria tests with the microscopic examination as the gold standard for diagnosis. Between January 2019 to February 2020, blood samples were collected from 360 patients, attending the internal medicine department at King Faisal Medical Complex at Taif, Saudi Arabia. Microscopy, CareStart™ and Optimal Malaria tests were done, and the accuracy was evaluated. In this study, 39 (10.6%) malaria positive slides were diagnosed, while 37 (10.2%) positive samples were detected with CareStart™ and Optimal Malaria tests. CareStart™ Malaria Test and Optimal Test gave (94.8%) sensitivity, (98.4%) specificity, (88.09%) positive predictive value, (99.3%) negative predictive value, and (98.6%) diagnostic accuracy. CareStart™ Malaria Test is considered one of the options in health areas especially those with limited laboratory functions despite their diagnostic accuracy problems. However, this study showed that the specify of CareStart™ Malaria Test is not always satisfactory when performed in clinical laboratories and so must be confirmed with other tests. Infections were significantly more in males than in females, (P<0.01). Malaria was primarily attributed to *Plasmodium falciparum* (*P. falciparum*) mono-infections, followed by *P. vivax* mono-infections, and lastly to mixed infections accounting for 24 (61.5%), 12 (30.7%) and 2 (5%) of microscopy confirmed malaria cases. 95% was the overall percent agreement between microscopy and the RDTs.

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Correspondence:
Khadiga Ahmed Ismail, Department of Applied Medical Science, Taif University, Saudi Arabia, E-mail: khadigaahmed68@yahoo.com

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Introduction

Malaria is a very serious, sometimes fatal, parasitic disease that is caused by a parasite that is transmitted between humans by the bite of infected Anopheles mosquitoes. Malaria is characterized by fever, chills, and anemia. There are four species of *Plasmodium* that can infect humans which are: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. In human, the infective stage is called a sporozoite, which migrates to the liver where it matures and is releases in another form, the merozoite. Malaria is a major health concern in many of the tropics and subtropics and infects more than 200 million humans in the world [1].

At the present, malaria is diagnosed by looking for the parasites in a drop of blood. Blood is put on a microscope slide and stained so that the parasites will be visible under the microscope. A recent clinical diagnostic issue related to malaria is the detection of malaria antibodies in human blood or serum samples by immunoassay. The ELISA format and Immunochromatographic (ICT) format (rapid test) to detect antibody of malaria are recently available. The key for effective

management of malaria cases is rapid and accurate diagnosis in order to start the management early and reduce the morbidities and the mortalities caused by delayed or poor management of patients [2].

Presumptive diagnosis of malaria through the presence of fever alone results in the overuse of antimalarial medications. Fever paroxysms, the hallmark of malaria, occur when infected RBCs rupture and release parasite-derived molecules that result in the production of pro-inflammatory cytokines by the host [3]. Under ideal circumstances, any clinical suspicion of malaria would be confirmed by a laboratory test that is simple to perform, rapid, sensitive, specific, and cheap. Hence, traditional malaria diagnosis by the examination of stained blood smears under the light microscope remains the gold standard for malaria diagnosis. Additionally, microscopy can routinely detect low levels of parasitemia as low as 40 parasites/µl, and even as low as 5 to 10 parasites/µl of blood in cases of experienced microscopists [4]. However, it is labor-intensive, time-consuming, requires technical expertise and the availability of a good quality microscope. Therefore, multiple alternative methods have been developed for the diagnosis of malaria including ICT assays and molecular amplification methods. Each of these methods has cons and pros in terms of test parameters, cost and technical complexity [5-7].

Rapid antibody tests for malaria are commercially available and most of them are ICT dipstick assays that detect *Plasmodium* antigens in the blood flowing along a membrane containing specific anti-Plasmodium antibodies. This ICT assay is accurate, rapid, and easy to perform and interpret. Moreover, it can be a useful method for the diagnosis of malaria in countries where two *plasmodial* species are co-endemic and where laboratory support is limited. Its sensitivity has been proved to be similar to the sensitivity of microscopy in both developing and developed countries, so the aim of this study is to evaluate CareStart™ and Optimal Malaria tests in the diagnosis of malaria.

Subjects and Methods

Descriptive study was conducted on 360 patients admitted to King Faisal Medical Complex (KFMC) internal medicine department complaining of fever >38°C and were clinically suspected to be malaria infection. 268 (83%) were males and 62 (17%) were females, with the age range between 20 to 45 years, in the period from January 2019 to February 2020. Most patients were workers from outside KSA. Blood samples were withdrawn by vein puncture collected on EDTA tubes to be tested for malaria by direct microscopy and RDTs. The blood samples processed within short time after withdrawal 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* spp. parasites, also optimal test and CareStart™ Malaria RDTs 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 l000 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. ovaleor* mixed infection. The density of infection was evaluated in 100 fields of thin films against the leucocytes counts taken from records of the patients to check the parasitemia, The following equation was used to calculate the number of parasites/µl = total parasite count/ WBC count X the total leukocyte count/µl.

Figure 1: Sex distribution of the patients under study.

Optimal test

Optimal-IT® malaria test (Diamed, Flow Inc. Portland, Oreg.) was performed according to the manufacturer' instructions. Briefly, to a well in a microtiter plate a drop of blood was added and mixed with two drops of lysis buffer A, to lyse the red blood cells and releases the pLDH. The specimens were migrated to the top of the strip. After ten min, the strips were placed in washing buffer B, to clear the hemoglobin from the strip. Positive and negative control samples were included with each batch tested. The entire process took approximately 20 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. ovale*, or *P. malariae*). When a third line was also presented, this indicated a positive response for *P. falciparum* infection.

CareStart™ malaria RDTs

This test was performed according to the manufacturer' instructions. Briefly, all kit components and specimen were kept at 10 min 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 collect the blood sample (5 µl) using a pipette provided or micropipette. Add 5 µl of whole blood into the 'S' well. Add 60 µl assay buffer solution (3 drops for vial type or 2 drops for bottle type) into the "A" well. Start a timer. Read result in 20 min. Reading of the result was avoided after 30 min 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 or P.f) 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 analysis was conducted using SPSS software, version 22.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Out of 360 clinically suspected cases, microscopy confirm that 39 (10.8%) were positive for *Plasmodium* infection by using microscopy, and of these 24 (61.5%) were infected by *P. falciparum*, 12 (30.7%) by *P. vivax*, 1 (2.8%) by *P. ovale* and 2 (5%) were mixed infections: (*P. vivax* and *P. falciparum* and *P. vivax* and *P. ovale*). Correspondingly, both Optimal test and CareStart™ Malaria RDT for detection of malaria infection in 37 (10.2%). The differences in detection rates of these diagnostic tests were not statistically significant (P>0.05). Using

Table 1: Validation of Optimal test results for diagnosis of malaria using direct microscopy as gold standard.

Applied tests	Direct microscopy			Sens.	Spec.	PPV	NPV	DA
	+ve (%)	-ve (%)	Total (%)	%	%	%	%	%
OptiMaL								
Positive	37(94.8%)	5(1.5 %)	42(11.6 %)	94.80%	98.40%	88.09%	99.30%	98.60%
Negative	2(5.2 %)	316(98.5%)	318(88.4 %)					
Total	39(10.9%)	321(89.1 %)	360(100 %)					

Sens.% = Sensitivity%, Spec.% = Specificity%
Optimal *vs.* direct microscopy, P> 0.05 = no significant difference.

Table 2: Validation of and Care Start™ Malaria RDT results for diagnosis of malaria using direct microscopy as gold standard.

Applied tests	Direct microscopy			Sens.	Spec.	PPV	NPV	DA
	+ve (%)	-ve (%)	Total (%)	%	%	%	%	%
Care Start								
Positive	37(94.8%)	5(1.5 %)	42(11.6 %)	94.80%	98.40%	88.09%	99.30%	98.60%
Negative	2(5.2 %)	316(98.5 %)	318(88.4%)					
Total	39(10.9%)	321(89.1%)	360(100 %)					

Sens.% = Sensitivity%, Spec.% = Specificity%
Care Start™ Malaria RDT *vs.* direct microscopy, P> 0.05 = no significant difference.

Table 3: Results of microscopy and RDTs for detection of malaria species.

	P. vivax	*P. falciparum*	*P. Ovale*	Mixed	Negative	Total Positive
Direct microscopy	12(30.7 %)	24(61.5 %)	1(2.5%)	2(5 %)	321(89.2 %)	39(10.8 %)
OptiMAL	13(35.1 %)	21(56.7 %)	1(2.7%)	2(5.4 %)	323(89.7 %)	37(10.2 %)
Care Start	12(32.4 %)	22(59.4 %)	1(2.7%)	2(5.4 %)	323(89.7 %)	37(10.2 %)

direct microscopy as gold standard, optimal test showed 5 (12.8%) false-positive samples that were negative by microscopy and 2 (5%) false-negative samples that were positive by microscopy (Table 1 and 2). At the same time, CareStart™ Malaria RDT showed 5 (12.8%) false-positive and 2 (5%) false-negative results. CareStart™ Malaria RDT showed a sensitivity (94.8%), specificity (98.4%), positive predictive value (88.09%), negative predictive value (99.3%) and diagnostic accuracy (98.6%) as well as Optimal test.

Discussion

Malaria is one of infectious diseases of tropical and sub-tropical countries, four countries have eliminated malaria and been certified by the World Health Organization (WHO) as malaria free. Nowadays, around thirty-four countries are in active phase of malaria elimination as well as the Kingdom of Saudi Arabia trying to eradicate malaria. Malaria control program in Saudi Arabia began in 1948 by the Arabian American Oil Company (ARAMCO) in the Eastern province, primarily to protect employees living around the oases. This program was used by the Saudi Arabian government as the template for a national malaria programs in 1952, which targeted malarious districts across the kingdom and was, designed to protect pilgrims en route to the holy sites of Mecca and Medina. Saudi Arabia joined the WHO global malaria eradication effort in 1963 and, by the early 1970s, transmission was arrested in the Eastern and Northern provinces. In Saudi Arabia, malaria persists in the provinces of Aseer and Jazan, both bordering the Republic of Yemen, following a series of out breaks of which the worst was in 1998 [8].

Malaria RDTs are immunochromatographic tests which designed to detect parasite antigens in whole blood samples. RDTs have many advantages over clinical diagnosis or microscopy. Infield trials, with ≥ 200 parasites/μL parasitemia they have demonstrated ≥ 90% sensitivity and specificity for *Plasmodium* spp. infection. They have many advantages such as simple and safe time in comparison with other malaria diagnostic tests, as they do not require expensive equipment. The results appear within 15 min to 20 min, and they are relatively inexpensive. RDTs are performed in a simple way, and no need for laboratory experts as its interpretation is so simple that clinicians or community health workers can be taught to perform them with a half day or full day of practical training. Correct interpretation of RDTs is less subjective than that of microscopy the test line is either present or absent. Currently available RDT products detect one or more of three target antigens. As RDTs have a shelf-life of about 18 to 24 months, this give sufficient time for its delivery, distribution, and use inmost settings but they need suitable temperature and humidity during their storage and shipping as describe by the manufacture. Because of their ease of use and accuracy, RDTs have an increased capacity to provide malaria diagnosis in nearly all healthcare settings, an essential component of accurate disease surveillance in an elimination setting [9].

Our study showed the sex distribution involved in the study and demonstrated that males are more affected with malaria than females as shown in Figure 1, our result is in agreement with study done by Ismail et al. [10] this may be due to men working in the fields are more exposed to mosquito bites. In some societies, men have a greater occupational risk of contracting malaria than women if they work in mines, fields or forests at peak biting times, or migrate to areas of high endemicity for work, in addition to leisure activities, sleeping arrangements may also affect malaria transmission. In some societies, men tend to sleep outdoors, and this may increase their risk of exposure to mosquitoes' bites [10]. Also, study done by Garley et al. [11] showed men may be more vulnerable than women to exposure, women may be more willing than men to invest in malaria prevention measures such as insecticide treated bed nets.

Our study showed that both CareStart™ and Optimal Malaria Test sensitivity (94.8%), specificity (98.4%), positive predictive value (88.09%), negative predictive value (99.3%) and diagnostic accuracy (98.6%) this in agreement with a study done by Abdel-Wahab et al. [12].

Our study showed 61.5% of diagnosed malarial cases were *P. falciparum* infected cases as shown in Table 3 this in contrast to study done by Abdel-Wahab et al. [12] as most of our patients are from Africa where *P. falciparumis* endemic.

Conclusion and Recommendation

RDT is very effective, rapid and simple method of diagnosis of malaria especially in non-endemic area like Saudi Arabia. It is recommended to consider good storage condition of CareStart™ Malaria RDT and Optimal kit to obtain accurate results.

Limitations and Interferences of Carestart™ Malaria RDT

This test is designed to detect HRP2 and pLDH antigens of Malaria Plasmodium species. Other clinically available tests are required if they obtained results are questionable. A definitive clinical diagnosis should not be made solely based on the result of this test but should only be made by a qualified physician after all clinical and laboratory findings have been evaluated. A positive result with faint test line or a false negative is possible due to a low parasite density. The pro zone effect may cause a false-negative result if the infecting parasite is *P. falciparum* containing HRP2 antigen. The test may produce a false positive result for a patient with acute schistosomiasis or a high level of rheumatoid factor.

Ethical Consideration

Ethical approval for this study was obtained from the Ethics Review Committee of the College of Applied Medical Sciences at Al-Taif University. Moreover, written consent before participation in the study was taken from each participant.

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