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Evaluation of sensitivity and specificity of immunochromatographic test for detection of malaria parasites *P. falciparum / P. vivax* in Sudan

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Abstract
Malaria diagnosis has received special interest from the researchers worldwide due to the challenges that faced Malaria control as the diagnosis considered the corner stone in malaria control. To counteract the drawbacks of the traditional methods used in malaria diagnosis, Immunochromatographic test has been extensively used nowadays. In this study we aimed to evaluate the sensitivity and specificity of Immunochromatographic test for detection of Malaria (*P. falciparum / P. vivax*) in patients with presumptive clinical diagnosis of Malaria in Sudan.
387 blood samples were tested using Immunochromatographic test and microscopic test, we found that 200 samples were negative, and 187 were positive. We used expert lab technicians who were blinded to the Immunochromatographic test result to prevent bias.
The overall sensitivity test of Immunochromatographic test was 98.91%, which is considered slightly low compared to the sensitivity value mentioned by the manufacturer (99.7%), but it is acceptable to detect *P. falciparum* parasitemia at densities of (11-100 parasite/100 field) and (1-10 parasites/field). The overall specificity was 67.45% which is very low compared to the specificity value mentioned by the manufacturer (99.3%). Therefore, Immunochromatographic test available in Sudan should be considered as a supportive tool to microscope in the diagnosis of Malaria and it can be used when the microscope is not available or guaranteed.

Keywords: Evaluation, immunochromatographic test, *P. falciparum/P. vivax*, Sudan

1. Introduction
The corner stone of malaria control is the accurate diagnosis; the main drawback of traditional malaria diagnosis method is the delay of obtaining results from the examination of blood samples from patients with suspected acute malaria make some of the more sensitive methods for malaria diagnosis impractical for routine laboratory use [1] so prompt and reliable diagnosis method is essential for a reduction of the morbidity and mortality of malaria [2]. Diagnosis of Malaria in Sudan is confirmed by two procedures, microscopic examination of blood films and antigen-based rapid diagnostic tests [3]. Although polymerase chain reaction-based tests have been developed, but they are not widely used due to their complexity [4]. Although microscopic examination is considered as a gold standard but its accuracy of the result depends on laboratory technician skills and parasite levels, beside the lack of equipment used for the test in many rural settings. In optimum conditions, the sensitivity of blood films should be in range of 75%-90%, to as low as 50%. [5]. In a study done in 2009, they evaluated practitioners' clinical and microscopists' technical skills in the diagnosis of malaria in central Sudan. In a retrospective study, 3203 blood smears from 95 peripheral health facilities (each represented by a general practitioner (GP), and general microscopist (GM) and were reexamined by expert microscopist. Furthermore, in prospective study 410 patients had their malaria diagnosis rechecked by rapid diagnostic test for validation of the microscopic diagnosis. Results showed that the rate of false-positive diagnosis of malaria was 75.6% and false-negative diagnosis was 0.01%. The study disclosed poor skills of the GP's and GM's in malaria diagnosis because 43% of the GP's and 44% of the GM's failed to make a single true-positive malaria diagnosis [6]. So Immunochromatographic test (ICT) for malaria diagnosis is nowadays extensively used to fill the gap [3]. So in this study we aimed to evaluate the sensitivity and specificity of the commercially available ICT for detection of Malaria Parasites antigen of P. falciparum / P. vivax compared microscopic result.

A lot of studies have evaluated the efficiency and performance of ICT in detecting malaria parasites and they reported that there were high variability in their sensitivity and specificity. One study conducted in Japan 2004 using animal model reported that ICT can't detect malaria parasite when the level of parasitemia is less than 2000 parasite/ µL [2]. Another study in Thailand conducted by E Russell and his colleagues found that When P. falciparum parasitemia rates were < 500/µL, the sensitivity of the diagnosis was only 23.3% [7]. The ICT test detected Plasmodium spp. infection with a sensitivity of 81.5% and a specificity of 81.9% was reported by P. Jos´e-Luis and his colleagues in Equatorial Guinea [8]. Research conducted in Indonesia by group of researcher reported that the sensitivity of the ICT for the diagnosis of *P. vivax* malaria was 96% with parasitemia of >500/ml but only 29% with parasitemia of <500/ml [9]. In Pakistan H, S Mahadev reported that ICT sensitivity for detection of *P. falciparum* was 97.0% and the specificity was 98.3% [10]. Another studies showed that the sensitivity and specificity were 100% and 84.5%, respectively [11].

2. Material and Methods

A laboratory based cross-sectional descriptive study, 387 blood samples collected from patients of presumptive clinical diagnosis of Malaria of all ages conducted in different hospitals in Khartoum, Sudan between March 2016 to June 2016. The material used were the ICT strips (The ICT compo cassette used is certified by ministry of health), buffer solution, capillary tubes, microscope (Olympus), thick blood smears, EDTA containers and Giemsa stains (RAL). Following the guideline of ministry of health of Khartoum state and all patients signed informed consent prior to study participation, 5 micro liters of blood was collected by finger prick that was already taken from patient by the lab technician for microscopic test. All specimens were tested with microscope by expert lab technicians, whom were blinded to the ICT result to prevent bias. Thick blood films were prepared and stained with Giemsa stain. The microscopist counted 200 white blood cells (WBC) before classifying a slide as negative. Parasite densities were calculated according to the standard method (parasite/µl = no. of asexual parasites × WBC count/no. of WBC counted). The data was analyzed using personal computer and statistical package for social sciences (SPSS).

3. Results

Table 1: illustrate the distribution of sample according to the microscopic result.

Microscopic result	Frequency	Percent
Positive	187	48.3%
Negative	200	51.7%
Total	387	100.0%

Table 2: illustrate the distribution of sample according to parasite count (range) in microscopic result.

Parasite count (range) in microscopic result	Frequency	Percent
(1-10) asexual parasites per 100 field	105	27.1%
(11-100) asexual parasites per 100 field	41	10.6%
(1-10) asexual parasites per field	41	10.6%
N/A	200	51.7%
Total	387	100.0%

Table 3: illustrate the distribution of sample according to ICT Result.

ICT Result	Frequency	Percentage
Positive	92	23.8%
Negative	295	76.2%
Total	387	100.0%

Table 4: illustrate the association between level of parasitemia in microscopic result and ICT Result.

Parasite count (range) in microscopic result		ICT Result		Total
		Positive	Negative	
(1-10) asexual parasites per 100 field	Count	23	82	105
	% within ICT Result	25.3%	85.4%	56.1%
(11-100) asexual parasites per 100 field	Count	27	14	41
	% within ICT Result	29.7%	14.6%	21.9%
(1-10) asexual parasites per field	Count	41	0	41
	% within ICT Result	45.1%	0.0%	21.9%
Total	Count	91	96	187
	% within ICT Result	100.0%	100.0%	100.0%

The overall specificity of the test was found to be 67.45% according to equation: D/ (D+B)*100, and the overall sensitivity was found to be 98.91% according to equation: A/(A+C)*100. When A = true positive sample, B = false negative samples, C = false positive samples and D = true negative samples.

Table 5: illustrate the association between Microscopic results and ICT Result.

Microscopic result		ICT Result		Total
		Positive	Negative	
Positive	Count	91	96	187
	% within ICT Result	98.9%	32.5%	48.3%
Negative	Count	1	199	200
	% within ICT Result	1.1%	67.5%	51.7%
Total	Count	92	295	387
	% within ICT Result	100.0%	100.0%	100.0%

4. Discussion

The overall sensitivity of ICT to detect *P. falciparum* was 98.9% which is less than that stated in the malaria ICT tested (99.7%) but within the range that reported by some of the previously mentioned studies [7, 9] during this study, *P. vivax* was not detected which assures the fact that *P. vivax* is rare in Sudan [2, 9]. Moreover, the overall specificity was 67.45%, which is weak compared to the specificity value of Malaria ICT tested (99.3%). low specificity rendered ICT less reliable in detecting malaria parasite and hence considered as a supportive tool in malaria diagnosis. Our results were different from the study that showed that the sensitivity and specificity 100% and 84.5%, respectively [11]. The ICT was found to have less capacity in predicting the presence of the disease, which demonstrates the weakness of the test in the diagnosis of Malaria, this is similar to the result of a published article from Equatorial Guinea 2010 by Portero JL *et al.* [8]. In agreement to our results all published

papers reported the low capacity of ICT to detect the *Plasmodium spp* at low parasitemia. The limitations of this study were the sample size and our failure to report the sensitivity and specificity of the ICT for *P. vivax* due to low prevalence of this parasite in Sudan.

5. Conclusion and Recommendation

In Sudan, the Immunochromatographic test for Malaria (*P.falciparum/ P. vivax*) has proven to be acceptable to detect parasitemia at densities more than 10 parasites/ 100 fields, therefore it can be used as a supportive diagnostic tool after microscopic test, in areas where microscope is not available, or when there is no microscope expertise. In parasitemia at densities less than 10 parasites/ 100 fields, ICT can't detect Malaria parasite, therefore microscopic tests should be used, and also we can conclude that the sensitivity and specificity of ICT depends on the level of parasitemia and the manufacturers. We recommend that the Level of parasitemia and all other factors that can affect the microscopic result against which the ICT is tested should be standardized in order for the manufacturers to synthesize internationally standard ICT, also there are a lot of factors can influence the reliability of these immunochromatographic tests must be taken into account such as exposure to high temperatures during transport and storage and the reagent used to enhance RBCs lyses.

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7. Declaration of interest: Authors declared that there is no conflict of interest in this study.

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