



CLINICAL EVALUATION OF RAPID DIAGNOSTIC KITS FOR MALARIA INFECTION

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ABSTRACT

Malaria is a mosquito-borne tropical or subtropical disease caused by *Plasmodium* parasite. Malaria rapid diagnostic tests (RDTs) represent one diagnostic method that is used in a variety of contexts to overcome limitations of other diagnostic techniques. In this study, we evaluated the accuracy and precision of the Malaria RDT kits (G Company, Korea) relative to the manufacturer's specifications in Korea, and Vietnam. 172 of RDTs were equally sensitive in detecting *Plasmodium falciparum*. 165 samples were positive and seven samples were negative. Specificity of G kits was 100.0% (172/172) for *P. falciparum* and its sensitivity was 95.9% with Pf/Pv Ag. RDT specificity was similar for detection of *P. vivax*. For detecting *P. vivax* infection, all 205 positive RDTs had similar sensitivity. Sensitivity for *P. vivax* was 95.9%. Among the 387 RDT negative samples, only five presented LDH band (plus HRP2 band), giving an overall LDH negativity rate of 98.7%. This helps to ensure the accuracy of the diagnosis, and the safety of the health worker and patient.

KEYWORDS: Malaria, *Plasmodium falciparum*, *P. vivax*, rapid diagnostic tests (RDTs).**INTRODUCTION**

Malaria is typically transmitted through the bite of an infected Anopheles mosquito. Infected mosquitoes carry the *Plasmodium* parasite. For many years, the global response to malaria was considered one of the world's great public health achievements.^[1] In 2016, 91 countries reported a total of 216 million cases of malaria, an increase of 5 million cases over the previous year.^[1] The global tally of malaria deaths reached 445 000 deaths, about the same number reported in 2015. Malaria is a mosquito-borne infectious disease affecting humans and other animals caused by parasitic single-celled microorganisms belonging to the *Plasmodium* group.^[1] Five species of *Plasmodium* can infect and be spread by humans.^[2] Most deaths are caused by *P. falciparum* because *P. vivax*, *P. ovale*, and *P. malariae* generally cause a milder form of malaria.^[2]

Malaria should be considered promptly in order to treat the patient in time and to prevent further spread of infection in the community via local mosquitoes. Malaria parasites can be identified by examining under the microscope a drop of the patient's blood, spread out as a "blood smear" on a microscope slide.^[3] Malaria is conventionally diagnosed by microscopic examination of stained blood films using Giemsa, Wright's, or Field's stains.^[4] The different forms of the 5 malaria species; the different stages of erythrocytic schizogony, the endemicity of different species, the interrelation between

levels of transmission, population movement, parasitemia, immunity, and signs and symptoms; drug resistance, the problems of recurrent malaria, persisting viable or non-viable parasitemia, and sequestration of the parasites in the deeper tissues, and the use of chemoprophylaxis or even presumptive treatment on the basis of clinical diagnosis, can all influence the identification and interpretation of malaria parasitemia in a diagnostic test.^[5] Various test kits are available to detect antigens derived from malaria parasites. Such immunologic (immunochromatographic) tests most often use a dipstick or cassette format, and provide results in 2-15 minutes. These Rapid Diagnostic Tests (RDTs) offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not available. Malaria RDTs are currently used in some clinical settings and programs. However, before malaria RDTs can be widely adopted, several issues remain to be addressed, including improving their accuracy; lowering their cost; and ensuring their adequate performance under adverse field conditions.^[3] Parasite nucleic acids are detected using polymerase chain reaction (PCR). Although this technique may be slightly more sensitive than smear microscopy, it is of limited utility for the diagnosis of acutely ill patients in the standard healthcare setting. PCR results are often not available quickly enough to be of value in establishing the diagnosis of malaria infection.^[3]

Malaria RDTs increase the availability and feasibility of accurate diagnosis and may result in improved quality of care. Though RDTs are used in a variety of contexts, no studies have compared how well or effectively RDTs are used across these contexts.^[6] There is a need to advocate for the use of evidence-based malaria RDTs for clinical decision-making and for the development and implementation of a robust algorithm for two different species (*P. falciparum* and *P. vivax*). In this study, we evaluated the accuracy and precision of malaria relative to the manufacturer's specifications at Korea and Vietnam.

MATERIALS AND METHODS

Clinical specimens and Clinical evaluation of RDT

RDTs for the detection of malaria antigens are based on the immunochromatographic test principle. These RDTs capture parasite antigen from peripheral blood using monoclonal antibodies prepared against a target malarial antigen and conjugated to gold particles in a mobile phase.

Cryopreserved aliquots of infected blood were kindly provided by S. University Hospital in Korea, I. University Hospital in Korea, and Vietnam Malaysia Institute in Vietnam. Genomic DNA was isolated from cryopreserved laboratory lines or cultures by use of a method described elsewhere.^[7] The microtiter plate hybridization (MPH) method was performed for accurate diagnosis as described previously.^[8-10] Briefly, the *Plasmodium*-specific 18S rRNA gene was amplified from 10-ml blood samples by using a pair of universal primers, biotinylated MPH-1 (59-biotin-CAGATACCGTCGTAATCTTA-39) and MPH-2 (59-CCAAAGACTTTGATTCTCAT-39).^[10] The template for PCR amplification consisted of 20 ng genomic DNA. The PCR amplifications were performed in a reaction volume of 12.5 μ l containing 20 ng of genomic DNA, 5.0 pM primer, 10.5 μ l of Prefect Shot Ex Taq PCR premix (TaKaRa Co, Japan); containing 0.4 mM dNTP, 4 mM Mg²⁺, 1.25 units Taq DNA polymerase, and Orange G/Bromophenol Blue for electrophoresis.

The comparative analyzers were performed by G Malaria RDT kits (G Company, Korea). The G Malaria RDTs are rapid diagnostic tests that diagnose malaria infection from whole blood of patients in 20 minutes. A total of 387 isolations was attempted for all acute samples. Assays were performed according to the manufacturers' instructions. In brief, 100 μ L of whole blood or serum sample was transferred by pipette into the sample well of the freshly unpackaged test device. The appearance of the test and control lines after a specified migration time (15-20 minutes) indicated a positive result. All RDTs had a control line and a test line. For each RDT involving the interpretation of the presence of a line, two people read the results independently and concurred on a given call. The technicians carrying out the evaluation of the test articles were blind to the malaria-infection status of the panel of serum samples and interpreted the colour line

(browish-red) on the immunochromatographic strip. This kits are designed to train health workers in the safe and accurate use of malaria rapid diagnostic tests (RDTs).

Statistical analysis

Statistical analysis was performed with Statistica version 18 (StatSoft, Inc., Tulsa, OK).^[11] The *p* values less than 0.05 were considered statistical significance. Categorical variables between groups were compared by Fisher's exact test. The t-test was used for continuous variables.

RESULTS

A total of 764 patients with suspected malaria were enrolled in this study. 387 patients of them were negative and the other (377) were found to be infected with *Plasmodium* parasites by microscopy and PCR. A total of 377 prospective serum samples submitted for malaria *P. falciparum* and *P. vivax* were tested by the G malaria kits (Figures 1 and 2). The appearance of the control line alone indicated a negative result. The characteristics of the study population (*n* = 377 cases) that contributed acute plasma to the test panel is shown in Table 1. 172 of RDTs were equally sensitive in detecting *P. falciparum*. 165 samples were positive and seven samples were negative. Specificity of G kits was 100.0% (172/172) for *P. falciparum* and its sensitivity was 95.9% with Pf/Pv Ag. RDT specificity was similar for detection of *P. vivax*. For detecting *P. vivax* infection, all 205 positive RDTs had similar sensitivity. Sensitivity for *P. vivax* was 95.9%.

Among the 387 RDT negative samples, only five presented LDH band (plus HRP2 band), giving an overall LDH negativity rate of 98.7% (Table 2). This positivity rate was even less (1.3%) when parasite densities were less than 1000.

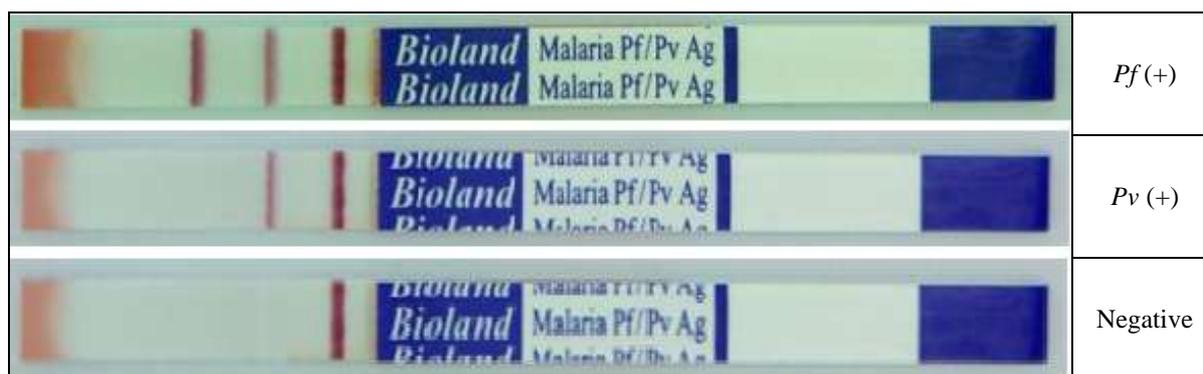


Figure 1: Positive: for malaria *P. falciparum* or mixed infection: In addition to the control line, two distinct pink - purple coloured lines appear at “Pf” and “Pv” sections of the test window.

Table 1: Clinical evaluation of specificity and sensitivity using the RDT-positive malaria in this study.

Total 377 Positives		<i>P. falciparum</i>		<i>P. vivax</i>	
		Positive	Negative	Positive	Negative
Malaria Pf/Pv Ag	Positive	165	0	190	0
	Negative	7	0	15	0
Total		172	0	205	0
Sensitivity		95.9%		92.7%	
Total Sensitivity		94.3%			

Table 2: Clinical evaluation of specificity and sensitivity using the RDT-negative malaria in this study.

Total 387 Negatives		Positive	Negative
Malaria Pf/Pv Ag	Positive	0	5
	Negative	0	382
Total		0	387
Sensitivity		98.7%	

DISCUSSION

Rapid diagnostic tests or RDTs are a way to test whether a person with malaria-like symptoms actually has malaria. Malaria is caused by a parasite that infects blood cells. The parasite is what causes the fever and other symptoms common to malaria.

Malaria rapid diagnostic tests (RDTs) assist in the diagnosis of malaria by detecting evidence of malaria parasites (antigens) in human blood. The utility of the detection of malaria RDTs as a recent infection indicator has already been demonstrated by some researchers.^[12-13] There are currently over 20 such tests commercially available (WHO product testing 2008).

RDTs commonly come in three different formats. The simplest form is a dipstick (test strips), which is placed in wells containing blood or buffer. The nitrocellulose strip may be placed in a plastic cassette or on a card. The *Pv* HRP2 test strips have 2 lines, one for the control which

is near to absorbing pads (below of Fig. 1) and the other for the *Pv* HRP2 antigen which is a pan-*Plasmodium* aldolase-PfHRP2 RDT (middle of Fig. 1). The PfHRP2/PMA test strips and the pLDH test strips have 3 lines (top of Fig. 1), first for control, and the other 2 for *P. falciparum* (PfHRP2 or pLDH, a pan-*Plasmodium* lactate dehydrogenase (pLDH)-*P. falciparum* histidine-rich protein 2 (PfHRP2) RDT specific for *P. falciparum*) and non-falciparum antigens (PMA or pan specific pLDH), respectively.

P. falciparum lactate dehydrogenase (PfLDH) is a 33 kDa oxidoreductase [EC 1.1.1.27].^[14] It is the last enzyme of the glycolytic pathway, essential for ATP generation and one of the most abundant enzymes expressed by *P. falciparum*.^[15] *Plasmodium* LDH (pLDH) from *P. vivax*, *P. malariae*, and *P. ovale* exhibit 90-92% identity to PfLDH from *P. falciparum*. Fructose-bisphosphate aldolase [EC 4.1.2.13] catalyzes a key reaction in glycolysis and energy production and is

produced by all four species.^[16] The *P. falciparum* aldolase is a 41 kDa protein and has 61-68% sequence similarity to known eukaryotic aldolases.^[17] Dzakah et al.^[18] elucidates the possibility of developing aldolase-specific RDTs which can differentiate the different *Plasmodium* infections and improve accurate diagnosis of malaria. Malaria CareStart™RDT test demonstrated a superior sensitivity compared to microscopy, which is the gold standard for malaria diagnosis.^[19] CareStart™RDT could be a useful tool in individuals suspected of malaria even in areas where prevalence is low. Senn et al.^[17] reported that treatment for malaria based on RDT results is safe and feasible even in infants living in areas with moderate to high endemicity for both *P. falciparum* and *P. vivax* infections. This results of the RDT kits of G Company could adequately differentiate between *P. vivax* and *P. falciparum* infections.

CONCLUSION

The commercially available malaria rapid diagnostic test (RDT) kit (G Company, Korea) was evaluated. A combination of both lactate dehydrogenase (PfLDH) and fructose-bisphosphate aldolase (PvALD) in RDTs for the rapid diagnosis of *P. falciparum* and *P. vivax* will enhance the sensitivity of the assay and reduce misdiagnosis.

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