Comparative Assessment of Diagnostic Performances of Two Commercial Rapid Diagnostic Test Kits for Detection of Plasmodium spp. in Ugandan Patients with Malaria

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Abstract: Prompt diagnosis of malaria cases with rapid diagnostic tests (RDTs) has been widely adopted as an effective malaria diagnostic tool in many malaria endemic countries, primarily due to their easy operation, fast result output, and straightforward interpretation. However, there has been controversy about the diagnostic accuracy of RDTs. This study was conducted to evaluate the diagnostic performances of the 2 commercially available malaria RDT kits, Rapigen Malaria Ag Pf/Pv (pLDH/pLDH) and Asan EasyTest™ Malaria Ag Pf/Pv (HRP-2/pLDH) for their abilities to detect Plasmodium species in blood samples collected from Ugandan patients with malaria. To evaluate the diagnostic performances of these 2 RDT kits, 229 blood samples were tested for malaria infection by microscopic examination and a species-specific nested polymerase chain reaction. The detection sensitivities for P. falciparum of Malaria Ag Pf/Pv (pLDH/pLDH) and Asan EasyTest™ Malaria Ag Pf/Pv (HRP-2/pLDH) were 87.83% and 89.57%, respectively. The specificities of the 2 RDTs were 100% for P. falciparum and mixed P. falciparum/P. vivax infections. These results suggest that the 2 RDT kits showed reasonable levels of diagnostic performances for detection of the malaria parasites from Ugandan patients. However, neither kit could effectively detect P. falciparum infections with low parasitaemia (< 500 parasites/μl).

Key words: Malaria, rapid diagnostic test, diagnostic performance, Uganda

INTRODUCTION

Malaria is the most prevalent parasitic disease in the world, with an estimated 3.2 billion people in danger of being infected and half of the world’s population at risk of contracting malaria [1]. In 2016, although the incidence of malaria has decreased in recent years, there were an estimated 216 million cases of malaria, an increase of about 5 million cases over 2015 and deaths reached 445,000, a similar number to the previous year [2]. Despite significant control efforts, morbidity and mortality induced by malaria remain high in many developing countries, especially in areas characterized by tropical and subtropical ecosystems. Among the 5 Plasmodium species of human malaria parasites, P. falciparum is the most dangerous and can cause severe clinical manifestations and even death [3]. P. vivax accounts for almost half of the malaria cases worldwide and is no longer considered as a mild infection [4]. Accurate malaria diagnosis is practically the only tool of effecting rational therapy. It is important to have sensitive and specific malaria diagnostic tools to prevent injudicious use of anti-malaria drugs and overtreatment. Microscopic examination of blood smears has been used as a gold standard for malaria diagnosis in many malaria endemic areas, despite its limitations on infrastructural and technical requirements that are not always available in resource-limited settings. Inconsistency due to intense inter-observer variability, particularly for samples with low parasitaemia or mixed Plasmodium species, has been regarded as a major shortcoming of microscopic examination [5-7]. These limitations have fostered the development of non-microscopic alternatives for the diagnosis of malaria, especially in field diagnosis. Although molecular methods based on polymerase chain reaction (PCR) allow for highly accurate diagnosis for malaria, these methods cannot be easily applied for point of care applications. Therefore, rapid diagnostic tests (RDTs) are
increasing their role as diagnostic aid in microscopic diagnosis of malaria in many endemic areas owing to their considerable advantages in field application [8,9]. The global availability and scale of use of RDTs has gradually increased over recent decades [10]. RDTs are immunochromatographic tests capable of detecting the antigens of one or more *Plasmodium* species. Although the use of malaria RDTs has improved global malaria control due to their ease of use and fast result interpretation, the accurate and adequate reporting of malaria cases for monitoring malaria trends remains a challenge in resource-limited nations [11]. In addition, commercially available RDTs do not present marked sensitivity over microscopic tools, given that their overall sensitivity is diminished when the parasitaemia level is low [12]. Moreover, their usefulness is limited owing to their inability to quantify parasitaemia. This study aimed to evaluate the quality of 2 commercially available malaria RDTs for their abilities to detect *Plasmodium* species in malaria cases from Uganda. Although their use in many public and private clinics in Korea and several countries has grown in recent years, their diagnostic performances in field settings have not been fully established, especially for clinical isolates from a variety of malaria endemic countries.

In this study, we assessed the diagnostic accuracy of these RDTs by comparing the sensitivity, specificity, and positive and negative predictive values of these RDTs with those of microscopic examinations and species-specific nested PCR in symptomatic Ugandan patients with malaria.

### MATERIALS AND METHODS

#### Ethics statement, study design, and population

All participants and patients were informed of this study and signed informed consent was obtained according to ethical standards. Prior to blood collection, applicable information was provided to patients, including information about the procedures themselves and the potential risks and benefits of this study. Young children under 8 years old, pregnant women, and patients with signs of severe and complicated malaria infections, as defined by WHO [13], were excluded from this study. This study was implemented in accordance with the principles of the Helsinki Declaration. Sampling was performed between April and September 2016 in primary health centers in the malaria endemic Kiyuni Parish of Kyankwanzi District, Uganda. Participants were sampled as previously described [14].

#### Microscopic examinations and nested PCR

The blood samples obtained from patients with malaria (*n* = 229) were screened by microscopy and species-specific nested PCR for species identification and determination of parasite density before performing the RDT examination. For microscopy examination, thick and thin capillary blood smears were prepared as previously described [14]. Smears were stained with a 4% Giemsa solution (pH 7.2) for 20 min. All smears were independently prepared by 3 trained Ugandan research technicians following the standard protocols [15,16]. The microscopic analysis was also confirmed by the Department of Tropical Medicine, Inha University College of Medicine to minimize diagnostic errors. Parasite density was calculated by counting against 299–500 white blood cells and estimated assuming 8,000 WBCs/μl [17]. After completing microscopic examination, all blood sample was used for PCR analysis. Genomic DNA was isolated from blood samples using QIAamp DNA blood kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. All samples from patients showing a positive microscopy result were further tested by species-specific nested PCR targeting *Plasmodium* 18S ribosomal RNA gene (18S rRNA), as described previously [5,18].

#### RDTs

Samples were used to detect malaria parasitaemia by blood smear and by nested PCR analysis. The RDTs used in this study were the RapiGEN Malaria Ag *Pf/Pv* (HRPII/pLDH) (Cat. No. H006087, RapiGEN INC, Gunpo, Korea) and the Asan Easy Test™ Malaria Ag *Pf/Pv* Strip (Cat. No. H2015, Asan Pharmaceutical Co. Ltd., Hwaseong, Korea). The used RDT kits were having the same Lot. No. for the accurate comparison. The Asan EasyTest™ Malaria Ag *Pf/Pv* (HRP-2/pLDH) detects the histidine-rich protein-2 (HRP-2) antigen specific for *P. falciparum* and *P. vivax*. The RapiGEN Malaria Ag *Pf/Pv* (pLDH/pLDH) detects various isomers of LDH antigens for *P. falciparum* and *P. vivax*. Each RDT test was performed following the manufacturer’s instructions. Test line intensities, which were visible as cherry-red to purple-colored lines, were scored according to each RDT as belonging to one of 4 categories: none (no line visible), weak (paler than the control line [+]), medium (equal to the control line [++]), and strong (stronger than the control line [+++]). All results were followed strictly adhering to the manufacturers’ instructions. There were no invalid test results. The evaluation process maintained an unbiased methodology. The status of
the samples was unknown to the persons involved in performing the pre-analytical and analytical procedures. To evaluate the specificity and sensitivity of each RDT on the basis of microscopic examination and nested PCR results, a study was performed on a sample panel consisting of 229 clinical samples collected from Ugandan patients with malaria.

**Data analysis**

The results from 2 different RDT kits were evaluated by microscopic examination as known as gold standard method of blood samples. *P*-values less than 0.05 were considered statistically significant. Sensitivity and specificity were calculated to assess the diagnostic performance of 2 RDTs compared to microscopy examinations and nested PCR. The sensitivity was defined as the percentage of positive results among samples confirmed to be positive by microscopic examinations. The specificity was the percentage of negative results among samples whose thick blood smears were negative.

**RESULTS**

Field tests were conducted in 6 villages of the Kiyuni Parish in Kyankwanzi District, Uganda in 2016. A total of 229 blood donor samples were obtained from the study participants. Of the 229 samples (the mean age was 21.13 ± 11.19 years old; male = 93; female = 136), 114 were negatives for both *P. falciparum* and *P. vivax* by microscopic examination and nested PCR analyses (Table 1). Expert microscopic examination de-

| Table 1. Comparison of diagnostic performance of two RDT kits with microscopic examination and nested PCR |
|----------------------------------------------------------------------------------|
| **Microscopy** | **PCR** | **RDT 1*** | **RDT 2*** |
| Neg. | Pf | Pf | Mixed** | Pf | Pf |
| Negative | 114 | 114 | - | 114 | 114 |
| Positive | 103 | 103 | 12 | 14 | 101 | 12 | 103 |
| Subtotal | 217 | 217 | 12 | 14 | 215 | 12 | 217 |
| Total | 229 | 229 | 12 | 14 | 227 | 12 | 229 |

*RDT1: Rapigen, Pf/Pv (pLDH/pLDH). RDT2: Asan EasyTest, Pf/Pv (HRP-2/pLDH).
**Mixed: Pf/Pv.*

| Table 2. Diagnostic performance of two RDT kits by parasitemia |
|---------------------------------------------------------------|
| **Parasites/μl** | **RDT Pf/Pv (pLDH/pLDH) (RapiGEN)** | **RDT Pf/Pv (HRP-2/pLDH) (Asan EasyTest)** |
| Weak | Medium | Strong | - | Total | Weak | Medium | Strong | - | Total |
| *P. falciparum* |
| >100,000 | - | - | 6 | 1 | 7 | - | - | 6 | 6 | 7 |
| 10,000-100,000 | 1 | 3 | 20 | - | 24 | 1 | 1 | 22 | - | 24 |
| 5,000-10,000 | - | 1 | 8 | 1 | 10 | - | 2 | 7 | 1 | 10 |
| 3,000-5,000 | - | 2 | 12 | 1 | 15 | - | 3 | 11 | 1 | 15 |
| 1,000-3,000 | 6 | 8 | 5 | 2 | 21 | 3 | 8 | 8 | 2 | 21 |
| 500-1,000 | 2 | 5 | 3 | 3 | 13 | 2 | 5 | 3 | 3 | 13 |
| 100-500 | 4 | 3 | 1 | 5 | 13 | 5 | 4 | 2 | 2 | 13 |
| <100 | - | - | - | - | - | - | - | - | - | - |
| Total | 13 | 22 | 55 | 13 | 103 | 11 | 23 | 58 | 11 | 103 |

**Mixed**

| Parasites/μl | **RDT Pf/Pv (pLDH/pLDH) (RapiGEN)** | **RDT Pf/Pv (HRP-2/pLDH) (Asan EasyTest)** |
|---------------|-----------------------------------|-----------------------------------|
| Weak | Medium | Strong | - | Total | Weak | Medium | Strong | - | Total |
| >100,000 | - | - | - | - | - | - | - | - | - |
| 10,000-100,000 | - | 3 | 3 | - | 6 | - | 4 | 2 | 6 |
| 5,000-10,000 | 2 | - | 1 | - | 3 | 1 | 1 | 1 | 3 |
| 3,000-5,000 | - | - | - | - | - | - | - | - | - |
| 1,000-3,000 | - | - | 1 | - | 1 | - | 1 | - | 1 |
| 100-1,000 | - | 1 | - | 1 | 2 | - | 1 | 1 | 2 |
| <100 | - | - | - | - | - | - | - | - | - |
| Total | 2 | 4 | 4 | 1 | 12 | - | - | - | - | 12 |

*negative.
detected 115 samples as being infected with *P. falciparum* (n = 103) or showing mixed infections of *P. falciparum* and *P. vivax* (n = 12). Microscopic examination and nested PCR analyses for 115 patients who tested positive for malaria suggested that *P. falciparum* infection was highly prevalent: *P. falciparum* infections (103/115, 89.57%) and mixed infections of *P. falciparum* and *P. vivax* (11/115, 10.43%). The mean age of the patients with malaria was 17.6 ± 10.69 years (Range: 2-61 years); females accounted for 58.83% of the total number of patients with malaria. Patients were distributed across all age groups, but the most highly represented patient group was the 10-19 year-old group (43/115 participants [37.39%]), followed by patients from the 0-9 [29/115 (25.22%)] and 20-29 (22/115, 19.13%) year-old groups. The geometric mean parasitaemia level was 19,948.56 parasites/μl; the maximum was 376,800 parasites/μl, and the minimum was 320 parasites/μl. The most prevalent parasitaemia group was the 10,000-100,000 (30/115) parasites/μl group, followed by the 1,000-3,000 (22/115) parasites/μl, 3,000-5,000 (16/115) parasites/μl, and 5,000-10,000 (13/115) parasites/μl groups. Blood samples from 114 participants were used to evaluate the specificity of the test kits. The mean age of the negative participants was 24.61 years (standard deviation: 10.08; range: 6-65 year of age) and comprised of 69/114 females (60.53%) (Table 2). Study participants belonged to all age groups, but the most prevalent participant group was that of the 20-29 year-old group (44/114 participants [38.60%]), followed by the 10-19 year-old group (33/114 [28.95%]), and the 30-39 year-old group (25/114 [21.93%]). The *P. falciparum* specificities for RapiGEN and Asan RDT brands were 100%. Overall, the *P. falciparum* sensitivities for RapiGEN and Asan RDT brands were 88% (101/115 patients), (specificity with 95% CI, Likelihood ratio+ [LR+]; 80%, Likelihood ratio- [LR-]; 93%) and 89.57% (103/115, Likelihood ratio+ [LR+]; 82%, Likelihood ratio- [LR-]; 94%) among the samples diagnosed as having *P. falciparum* and mixed malaria infections, respectively (Tables 1, 2). The *P. falciparum* specificities for RapiGEN and Asan RDT brands were 87.83% (101/115 patients) and 89.57% (103/115) among the samples diagnosed as having *P. falciparum* and mixed malaria infection, respectively (Tables 1, 2).

**DISCUSSION**

This study was conducted to measure the overall diagnostic performances of 2 RDT kits for *Plasmodium* infection. Patient blood samples were confirmed as either positive (115 patients) or negative (114 participants), in order to determine the appropriate test for field use Uganda. To be useful and provide an efficient diagnostic method, RDTs must exhibit greater than 95% sensitivity according to WHO recommendation [19]. Overall, the *P. falciparum* sensitivities for RapiGEN and Asan RDT brands were 87.83% (101/115 patients) and 89.57% (103/115) among the samples diagnosed as having *P. falciparum* and mixed malaria infection, respectively (Tables 1, 2). *P. falciparum* sensitivities for RapiGEN and Asan RDT brands were found to be 61.54% (8/13 patients) and 84.62% (11/13) for parasite density > 500 parasites/μl and 94.44% (93/90) and 90.00% (81/90) for < 500 parasites/μl, respectively. Similarly, the sensitivities for the mixed infection samples were 91.67% (11/12) for the 2 test kits. In addition, the sensitivity for the Asan brand kit was found to decline for *P. falciparum* at a parasite density < 100,000 parasites/μl. Intensities of test line with 10,000-1,000,000 parasites/μl densities were found to be reduced in the *P. falciparum* samples for the RapidGEN brand and in the *Pf* mixed samples for the Asan brand, respectively (Table 2). It is practically acceptable that comparative assessment is difficult because [1] reference standards are dissimilar; [2] clinical epidemiological characteristics of study populations; [3] trials do not employ common guidelines; [4] the timing of the study could influence the prevalence of malaria in the population; and [5] products of different lots may differ in quality [20,21]. According to previously published RDT study reports, HRP-2 assays commonly provide *P. falciparum* sensitivity of > 90% in clinical cases [22-24]. Study of HRP-2-based RDTs can be influenced by several factors, including antigenic variability of the target protein, antigen persistence in the bloodstream following elimination of parasites, and parasite density below the RDT threshold of detection [25-27]. Reassuringly, a recent trial evaluating 8 independent HRP-2-based RDTs in Western Kenya reported sensitivities that were comparable to microscopic examination of 90-95% [28]. In the case of pLDH assays, although product lots and variable field stability of the test kits could not be ruled out, the test results varied among studies. Sensitivity for *P. falciparum* is excellent (> 95%) in some studies and poorer (more than 80%) in other studies [12,29]. In general, it is acceptable that overall
RDT specificity is commonly observed to be 85% [12]. In this study, although there is a limitation that it has been done on a relatively small scale and in a limited area experiment, these RDT kits we have used could be a good alternative diagnostic tool, given the cost, technical, and local circumstances of PCR and microscopic examinations. However, it is also necessary to obtain the results of a study that can be generalized by examining and comparing different regions over a long period of time. In addition, compared to the other brand RDT kits that are now using for the diagnosis of *Plasmodium* species, there are same limitations of poor detection of mixed infection, of all species in *Plasmodium* and of the antigen in specimen continued after the treatment.

In conclusion, the present study reported the diagnostic performances of 2 commercially available malaria RDTs, Rapid-GEN Malaria Ag *Pf/Pv* (pLDH/pLDH) and Asan EasyTest™ MalariaAg *Pf/Pv* (HRP-2/pLDH), for blood samples collected from Ugandan patients with malaria. The overall diagnostic accuracy of the RDTs against *P. falciparum* was comparatively similar to that of RDTs recommended by WHO for a parasite density < 500. The sensitivity for samples with mixed infection was 91.67% for both RDTs. Therefore, the 2 RDT kits evaluated in this study appear to be relatively reliable diagnostic tools to detect *P. falciparum* infections in Uganda. However, further measurement is needed to fully evaluate the diagnostic performances of these 2 RDTs, including a more extensive examination of samples from various global regions and populations.

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**CONFLICT OF INTEREST**

The authors declare that they have no competing interests.

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