JITMM Proceedings
www.jitmm.com/proceeding
J I T M M Proceedings 11 (2023) 47-53

EVALUATION OF ULTRA-SENSITIVE RAPID DIAGNOSTIC TEST (US-RDT) FOR *PLASMODIUM FALCIPARUM* MALARIA

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ABSTRACT

Malaria has remained one of the critical parasitic diseases in the tropics. A precise and rapid diagnosis is required for early malaria detection and treatment. It reduces malaria morbidity and mortality. This study aimed to evaluate the performance diagnosis of *Plasmodium falciparum* histidine-rich protein2 (PfHRP2)– based ultra-sensitive rapid diagnostic test (US-RDT). Archived blood samples of severe malaria (N=100) and uncomplicated malaria (N= 20) were used to evaluate the performance of US-RDT compared to the gold standard by microscope. Cronbach's Alpha and Intra-class Correlation Coefficient (ICC), the percentage of agreement, were assessed from the results read by four independent observers. The results showed high reliability of the percentage of agreement as 0.927 for Cronbach's Alpha and 0.915 (0.875-0.943) with a 95% confidence interval for ICC. The sensitivity and specificity of US-RDT were quite high at 92.5% and 100%, respectively. Other essential measures of test performance, positive predictive value (PPV) and negative predictive value (NPV) were 1 and 0.53, respectively. In conclusion, US-RDT had high performance being used for malaria diagnosis in the field. The performance of the test depends on the level of HRP-2 in the blood. Other factors such as the deletion of the *Pfhrp2/3* gene need further investigation.

INTRODUCTION

Human malaria is caused by an organism in the phylum Apicomplexa which are protozoan belonging to the genus *Plasmodium* consisting of five species. However, *P. falciparum* and *P. vivax* are recorded to be the greatest public health challenge since they are causing the most death from malaria (Ashley *et al,* 2018). To achieve the global goal of malaria eradication by 2030, well-controlled transmission, and proper diagnosis are necessary for this destination. Rapid diagnostic test (RDT), a nitro-cellulose strip test that relies on the capture of dye-labelled antibodies, has been used as one of the malaria diagnosis methods over decades in small community to large scale settings. The RDT can differentiate the infecting species by specific antigen detection. The major antigens detected by RDTs are lactate dehydrogenase (pLDH), aldolase, and histidine-rich protein 2 (HRP-2) (Menegon *et al,* 2017). Lactate dehydrogenase and aldolase can be detected in all *Plasmodium* species while HRP-2 is specific only for *P. falciparum*. Most RDTs available therefore use HRP-2 as a target because its variation in the sequence affects the sensitivity of RDTs. However, it has been reported that in some circumstances, HRP-2-based rapid diagnostic tests are slightly sensitive, especially

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for parasites that express a small amount of the HRP-2 antigen or parasite with *pfhrp2* deleted gene generating false-negative results (Cowman *et al,* 2016). Likewise, the quantitation of HRP-2 concentrations in asymptomatic, uncomplicated and severe malaria seemed to be literally different as the geometric mean value (95% CI) of the protein antigen concentration increased with severity in contrast with peripheral parasitemia (Uyoga *et al,* 2021). In this study, the new ultra-sensitive rapid diagnostic tests (US-RDT) which have the ability to detect specific *P. falciparum* strains were evaluated. The performance of the test known as sensitivity, specificity, and predictive values was interpreted in this study.

MATERIALS AND METHODS

A pilot study was designed and conducted at the malaria research unit, Faculty of Tropical Medicine, Mahidol University. This study was a part of the multi-center clinical studies which has been approved by the Oxford Tropical Research Ethics Committee and the Central Research Ethics Committee (approval no. 014/2015). As the pilot study, the sample size was calculated; as N=73. However, the finite quantity of the US-RDTs was supported with limitation.

Sample preparation

In this study, the archived plasma samples were used to evaluate the performances of RDTs instead of whole blood samples due to the concentration of target antigen (HRP-2) in plasma directly effects the RDTs and to reduce the unwanted factors for dilution sample preparation. All archived plasma samples as a part of clinical trials (AQUAMAT) which has been approved from OXTREC ethic committee (*P. falciparum* severe malaria = 100, *P. falciparum* uncomplicated malaria =20) were thawed on ice and gently mixed by vortex until the sample is homogeneous. Samples were diluted with 0.01% PBS-T to 1:5, 1:10, and 1:20 (V/V) and mixed until homogeneously before testing. The plasma from healthy volunteer were used as the control (N=10).

Microscopic detection

The thick and thin blood films were prepared, fixed with methanol, stained with Giemsa solution and investigated using microscopic examination, The number of infected red cells was counted per 1000 uninfected red cells and report the results as parasitemia from the related samples were used to analyze the relationship between parasitemia and band intensity of all plasma samples, and the relationship between parasitemia and visual score of band intensity in all plasma samples.

US-RDT testing and readout

After each sample was mixed, 5 μl of plasma sample was added into a round specimen well. Then, the assay diluent was dispensed 4 drops into the square assay diluent well. Readout results at 20 minutes and record visual assessment score within 30 minutes by four independent observers. The score was rated as score level depending on the band intensity (table 1). The band intensity of the US-RDTs was also assessed by BioRad Gel DocTM XR+ (white light mode) for the digital analysis.

RESULTS

In this study, the results are divided into three parts; the agreement of consistency of reliability, the test performances and digital assessment for correlation analysis. The score levels rated by four independent observers of 100 archived plasma severe malaria samples with dilution zero (undiluted) at 20 minutes were evaluated to see the internal consistency of reliability and intra-class correlation. The percentage of agreement among four observers showed high internal consistency of reliability = 92.7% (Cronbach's alpha = 0.927) calculated by reliability analysis using SPSS program. And, also the intra-class correlation which showed 0.915 (0.875-0.943) with a 95% confidence interval for ICC. The test performances known as sensitivity and specificity were quite high with 92.5% and 100%, respectively. Other essential measures of test performance, positive predictive value (PPV) and negative predictive value (NPV) were 1 and 0.53, respectively (table1).

For the digital assessment of correlation analysis, the band intensity was assessed by using the colorimetric application of BioRad Gel DocTM XR+. All 130 samples were used in this analysis. The overall median (interquartile range) of band intensity (N=130) was 488,034 (13666.5-515704).

From table 2, the band intensity of the negative control (healthy) group was less than 0 after subtracting by the background. The median (interquartile range) of band intensity was 7,181 (-5783 – 107612) for uncomplicated malaria and 504,208 (451,606 – 521,718) for severe malaria. There was a significant difference in band intensity among the three groups (P = 0.001, Kruskal Wallis test). The band intensity of severe malaria was significantly higher than those of uncomplicated malaria and healthy group, P<0.01 (Mann-Whitney U test). There was no significance in band intensity between uncomplicated malaria and the healthy group, P=0.24, by Mann-Whitney U test) The relationship between parasitemia and band intensity of all plasma samples was investigated. As shown in Figure 1, there was no correlation between parasitemia and band intensity for all groups (r=- 0.18, P=0.04, Spearman correlation). In addition, there was no relationship between parasitemia and a visual score of the band in all groups ((r=- 0.13, P=0.16, Spearman correlation).

DISCUSSION

Malaria is still one of the most dangerous diseases in tropical areas and needed to be eradicated and eliminated. Although there is a

Table 1 Diagnostic performance of US-RDT in reference to microscope as gold standard.

Performance of US-RDT	All *P. falciparum* positive samples	Severe *P. falciparum* malaria	Uncomplicated *P. falciparum* malaria
Sensitivity	92.5%	100%	55%
Specificity	100%	100%	100%
PPV	100%	100%	100%
NPV	58.4%	100%	53%

Table 2 Baseline characteristics of band intensity of US-RDT

Plasma	Healthy	Uncomplicated malaria	Severe malaria
Sample (N)	N=10	N=20	N=100
Parasitemia (parasite/uL, -median (interquartile range)	-	177,710 (8,053-39,658)	920 (280-2,980)
Visual reading (positive/negative)	0/10	11/20	100/100
The intensity of sample' band* (reading unit, median (range)	871 (-6,711 to -4,307)	7,181 (-18,345 to 322,183)	504,208 (-9,668 to 569,268)

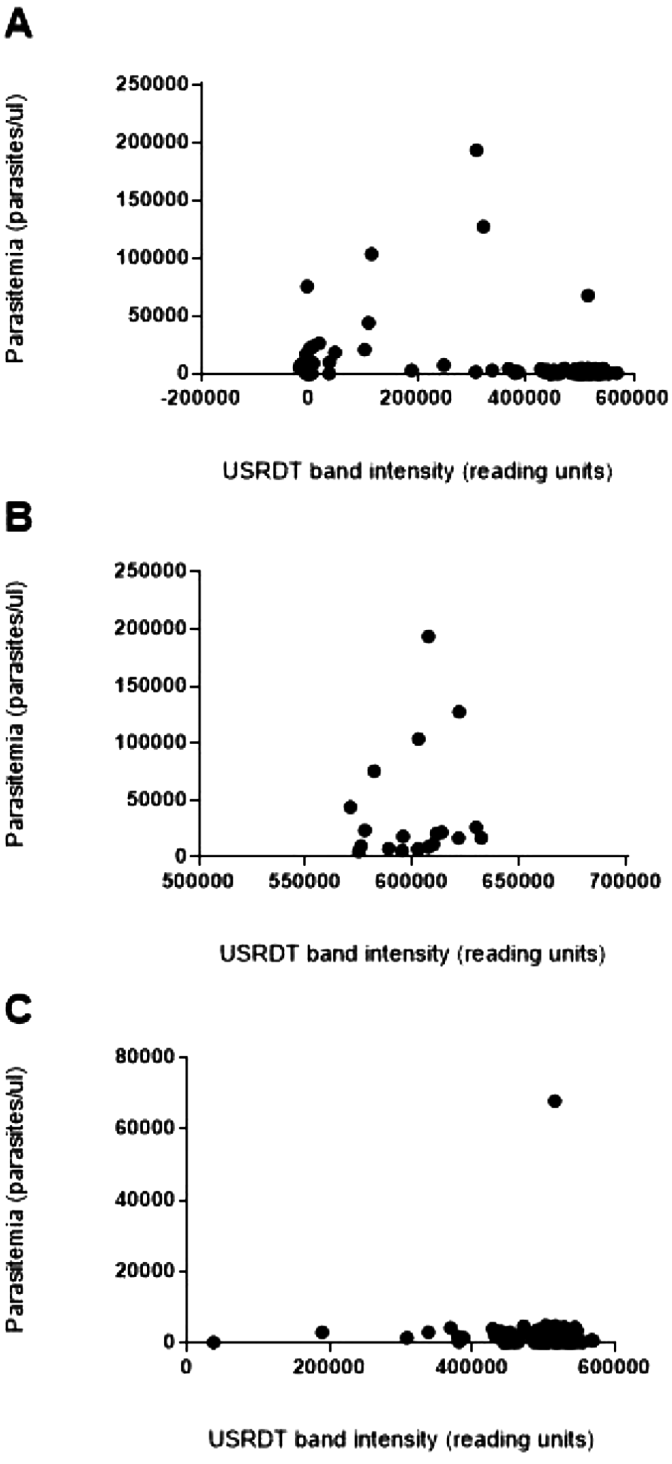

Fig 1 Relationship between parasitemia and band intensity of all plasma samples (A), uncomplicated malaria samples (B), and severe malaria samples (C).

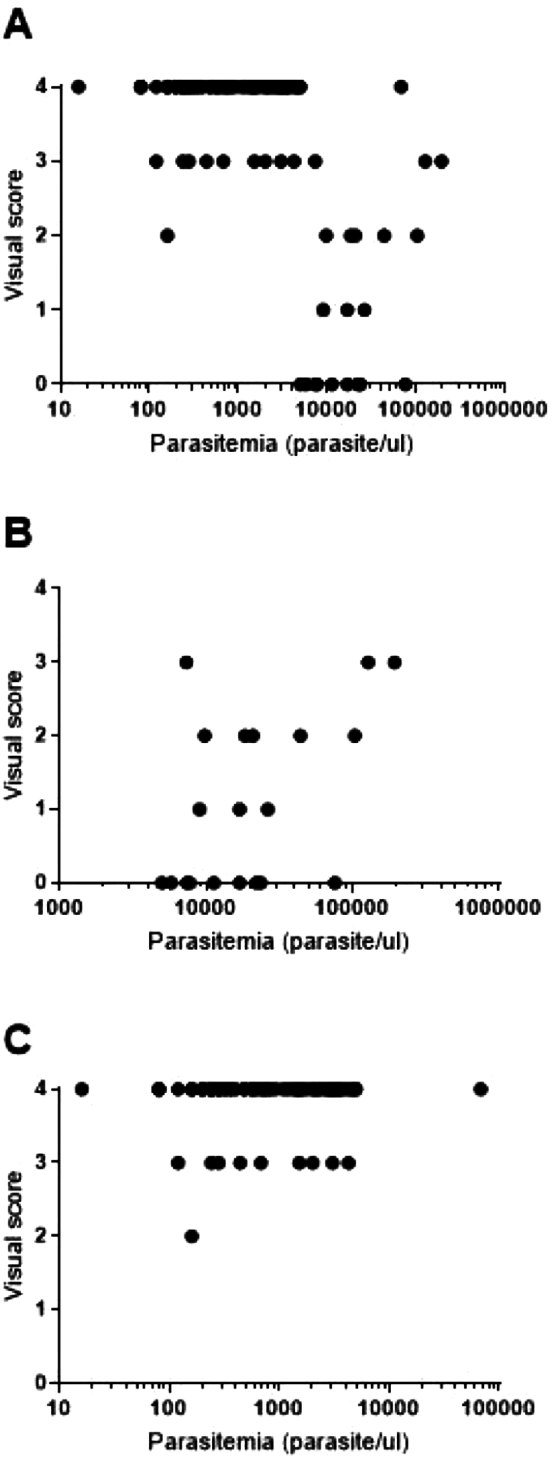

Fig 2 Relationship between parasitemia and a visual score of band intensity in all plasma samples (A), uncomplicated malaria samples (B), and severe malaria samples (C).

reduction in the transmission rate of *P. falciparum* in many endemic areas of the African region or arising in several countries that gradually declared as malaria-free, malaria has revealed a considerable presence of an asymptomatic infection that is undetectable by the current field (Phillips *et al,* 2017; Mbanefo and Kumar 2020). Nowadays, an up-to-date method used for malaria diagnosis is announced with more sensitivity to enhance the ability in submicroscopic or asymptomatic detection. With many techniques, Rapid Diagnostic Test (RDT) usually be the first option to be selected for disease screening in a resource limited setting or somewhere difficult to reach before treatment due to its advantages such as requiring less intensive of well-trained staff, affordable, easy to perform and give a rapid result within twenty minutes which is the remarkable strength of this technique, (Yeung *et al,* 2020). In this study, the newly developed ultra-sensitive rapid diagnostic test (US-RDT) is evaluated in a laboratory using the archived plasma malaria samples, which came from uncomplicated and severe falciparum malaria. To see the effectiveness of the test, the sensitivity, specificity, PPV, and NPV were investigated. We found that the performances of US-RDT were higher when tested with severe malaria samples than those with uncomplicated malaria samples. This might support the previous study told that using RDT for uncomplicated cases detection will be misdiagnosed the active cases with mild symptoms which will progress to severe in the future, (Elechi *et al,* 2015; Acquah *et al,* 2021). The possible explanation is that parasite biomass in severe malaria is significantly higher than those in uncomplicated malaria, (Dondorp *et al,* 2005). Parasite biomass reflected the parasite in the peripheral blood and sequester parasite. HRP-2 levels reflected parasite biomass, particularly in severe malaria, (Poti *et al,* 2020). We presented here, there was no correlation between parasitemia and US-RDT results. In addition, *pfhrp-2/3* gene deletion may result in the false-negative results of the US-RDT based on HRP-2. There was reported that HRP2-based RDTs were unable to detect *P. falciparum* infections in regions where the *hrp2* genes are deleted from a large proportion of the parasite population, (Lee *et al,* 2006; Gamboa *et al,* 2010). The combination of early and accurate diagnosis by RDT and early treatment with artemisinin-based combination therapy demonstrated the ability to reduce malaria morbidity and mortality in low-transmission settings, (Thang *et al,* 2009, Carrara *et al,* 2006). The interpretation of RDTs is less subjective than that of microscopy as it is easy to train and confident to report the results quickly (either present or absent of the line). In addition, the cost-effectiveness of malaria diagnosis by using RDTs seems to be lower compared to microscopic examination due to the type of instrument, maintenance process and no need of skilled microscopists where the resource is limited. However, there were some disadvantages of RDTs, particularly HRP2-based tests, which are highly sensitive for *P. falciparum* infections but not reliable to detect low parasitemia (<100 parasites/μL). The results of RDT based on PfHRP-2 were compared with ELISA in clinical sample. They found that plasma PfHRP2 could be assessed via a single RDT and assisted in patient management and clinical trials (Sinha *et al,* 2015). RDTs detection for other human species is under research and development.

ACKNOWLEDGEMENT

I am deeply grateful to Professor Kesinee Chotivanich and my co-advisors for their advices, assistance and encouragement throughout this research. Many thanks to malaria lab staff at MORU for their technical supports. This research is supported by the Faculty of Tropical Medicine, Mahidol University and Mahidol Oxford Tropical Medicine Research Unit (MORU).

REFERENCES

Acquah FK, Donu D, Obboh EK, Bredu D, Mawuli B, Amponsah JA, et al. Diagnostic performance of an ultrasensitive HRP2-based malaria rapid diagnostic test kit used in surveys of afebrile

people living in Southern Ghana. *Malar J*. 2021 Mar;20(1):125.

Ashley EA, Pyae Phyo A, Woodrow CJ. Malaria. *Lancet*. 2018 Apr;391(10130):1608-21.

Carrara VI, Sirilak S, Thonglairuam J, Rojanawatsirivet C, Proux S, Gilbos V, et al. Deployment of early diagnosis and mefloquine-artesunate treatment of falciparum malaria in Thailand: the Tak Malaria Initiative. PLOS Med. 2006 Jun;3(6):e183.

Cowman AF, Healer J, Marapana D, Marsh K. Malaria: biology and disease. *Cell*. 2016 Oct;167(3):610-24.

Dondorp AM, Desakorn V, Pongtavornpinyo W, Sahassananda D, Silamut K, Chotivanich K, et al. Estimation of the total parasite biomass in acute falciparum malaria from plasma PfHRP2. PLOS Med. 2005 Aug;2(8):e204.

Elechi HA, Rabasa AI, Bashir MF, Gofama MM, Ibrahim HA, Askira UM. Uncomplicated malaria in children: the place of rapid diagnostic test. Niger Med J. 2015 Mar;56(2):85-90.

Gamboa D, Ho MF, Bendezu J, Torres K, Chiodini PL, Barnwell JW, et al. A large proportion of *P. falciparum* isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. *PLOS ONE*. 2010 Jan;5(1):e8091.

Lee N, Baker J, Andrews KT, Gatton ML, Bell D, Cheng Q, et al. Effect of sequence variation in *Plasmodium falciparum* histidine-rich protein 2 on binding of specific monoclonal antibodies: implications for rapid diagnostic tests for malaria. *J Clin Microbiol*. 2006 Aug;44(8):2773-8.

Mbanefo A, Kumar N. Evaluation of malaria diagnostic methods as a key for successful control and elimination programs. *Trop Med Infect Dis*. 2020 Jun;5(2):102.

Menegon M, L'Episcopia M, Nurahmed AM, Talha AA, Nour BYM, Severini C. Identification of *Plasmodium falciparum* isolates lacking histidine-rich protein 2 and 3 in Eritrea. *Infect Genet Evol*. 2017 Nov;55:131-4.

Phillips MA, Burrows JN, Manyando C, van Huijsduijnen RH, Van Voorhis WC, Wells TNC. Malaria. *Nat Rev Dis Primers*. 2017 Aug;3:17050.

Poti KE, Sullivan DJ, Dondorp AM, Woodrow CJ. HRP2: transforming malaria diagnosis, but with caveats. *Trends Parasitol*. 2020 Feb;36(2):112-26.

Sinha I, Ekapirat N, Dondorp AM, Woodrow CJ. Use of a rapid test to assess plasma *Plasmodium falciparum* HRP2 and guide management of severe febrile illness. *Malar J*. 2015 Sep;14:362.

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 Jan;8(3):1-10.

Uyoga S, Wanjiku P, Rop JC, Makale J, Macharia AW, Nyutu GM, et al. Plasma *Plasmodium falciparum* histidine-rich Protein 2 concentrations in children with Malaria infections of differing severity in Kilifi, Kenya. *Clin Infect Dis*. 2021 Oct;73(7):e2415-23.

Yeung S, McGregor D, James N, Kheang ST, Kim S, Khim N, et al. Performance of ultrasensitive rapid diagnostic tests for detecting asymptomatic *Plasmodium falciparum*. *Am J Trop Med Hyg*. 2020 Feb;102(2):307-9.