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Performance of First Response® and CareStart™ Malaria Rapid Diagnostic Tests for the Detection of *Plasmodium falciparum* in a Tertiary Hospital in Ghana

Bernice Ameyaw[1], Mohamed Mutocheluh[1*], Michael Owusu[2], Alhassan Abiba Khalifah[3], Richard Vikpebah Duneeh[3] and Patrick Williams Narkwa[1]

[1]Department of Clinical Microbiology, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
[2]Department of Microbiology, Kumasi Centre for Collaborative Research in Tropical Medicine, Ghana.
[3]Department of Haematology, Komfo Anokye Teaching Hospital, Kumasi, Ghana.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Malaria poses a major public health problem in sub-Saharan Africa. In Ghana, millions of people are potentially at risk of *Plasmodium falciparum* infections annually. The current study evaluated the performance of two Histidine rich protein 2 (HRP-2) rapid diagnostic tests (First Response® and CareStart™) using giemsa stained microscopy (microscopy) as the gold standard. This cross-sectional study which took place at the Komfo Anokye Teaching Hospital

**Corresponding author: E-mail: mmutocheluh.chs@knust.edu.gh, mutocheluh@gmail.com;*

(KATH) in Kumasi from October 2014 to March, 2015 was done to monitor the performance of RDTs that are used regularly in Ghana.

Methodology: A total of 400 children (239 males, 161 females; age range 1-17 years) with fever or history suggestive of malaria were included in the study. First Response® and CareStart™ diagnostic accuracy results were compared with that of microscopy. The strength of agreements (kappa) between the microscopy and the two RDTs were also calculated.

Results: Of the 400 blood films that were examined using microscopy, *Plasmodium* parasites were detected in 33 (8.3%) of them. First Response® showed positive results in 65 (16.3%) and CareStart™ showed positive results in 68 (17.0%). The sensitivities of both First Response® and CareStart™ when compared with microscopy were 97.0% (95% CI: 84.2-99.9) and 97.0% (95% CI: 84.2-99.9) respectively. The specificities were First Response® 91.0% (95% CI: 87.6-93.7) and CareStart™ 90.2% (95% CI: 86.7-93.0). The strength of agreement (kappa) between microscopy and First Response® and CareStart™ with 95% confidence interval was good for the First Response® (giemsa stain microscopy vs First Response®: 0.61) and moderate for CareStart™ (giemsa stain microscopy vs CareStart™: 0.59).

Conclusion: The diagnostic accuracy of the First Response® and CareStart™ RDTs to detect malaria was good with no significant differences between the two rapid test kits when compared with microscopy. The RDTs are a suitable alternative to microscopy to test for malaria in rural areas.

Keywords: RDT; first response® and carestart™; plasmodium; malaria; microscope.

ABBREVIATIONS

Histidine rich protein 2 (HRP 2), Rapid Diagnostic Test (RDT), World Health Organization (WHO), Artemisinin-based Combination Therapy (ACT), Special Program for Research and Training in Tropical Disease (TDR), Foundation for Innovative New Diagnostics (FIND), and the Centre for Disease Control and Prevention (CDC), Paediatric Emergency Unit (PEU), Komfo Anokye Teaching Hospital (KATH), Accident and Emergency (A&E), Ethylenediaminetetraacetic acid (EDTA), Complete Blood Count (CBC), High power fields (HPF), White blood cells (WBCs), Red blood cells (RBCs), Positive predictive value (PPV), Negative predictive value (NPV), Plasmodium lactate dehydrogenase (pLDH), Plasmodium aldolase (pAldo), Polymerase chain reaction (PCR).

1. INTRODUCTION

Malaria is one of the commonest causes of febrile illness among children and adults in Ghana and other West African countries. In 2015, WHO estimated that the global incidence of malaria was 214 million cases with an estimated 438,000 malaria deaths [1]. According to WHO, the sub-Saharan Africa accounted for 89% of new malaria cases and 91% of malaria deaths in 2015 [1]. Malaria in humans is mainly caused by parasites such as *Plasmodium ovale, Plasmodium malariae*, *Plasmodium vivax* and *Plasmodium falciparum.* These parasites are usually transmitted through the bite of female mosquitoes that belong to the genus *Anopheles*. Interestingly, *Plasmodium falciparum* has been identified as the commonest and main cause of malaria in humans [1].

Most febrile illnesses are presumptively treated as malaria in Ghana and other countries in Africa even though there is increasing evidence to support the fact that malaria is just one of the many causes of febrile illnesses. For example, in a study conducted in Tanzania in which of 528 (60.7%) out of 870 patients who were diagnosed with malaria and treated with antimalarial drugs, laboratory tests confirmed that only 14 (1.6%) indeed had malaria [2]. Similarly, a study conducted in Accra the capital of Ghana reported that of 605 feverish children who sought care at a hospital in Accra, only 11% tested positive for malaria by microscopy after 80% had been diagnosed with malaria and treated with anti-malarial drugs [3].

The problem associated with presumptive diagnosis and treatment of malaria apart from safety and cost is the development of drug resistant strains. The development of drug resistant strains may also be linked to misuse of anti-malarial drugs. In Ghana anti-malarial drugs are sold over the counter a practice that encourages self-medication and may contribute to the development and spread of anti-malarial

drug resistance. The services of most laboratories in Ghana with reference to the diagnosis of malaria is limited to the microscopic examination of clinical samples. Microscopy requires laboratory facilities, is cumbersome in areas with unreliable power supply, is time consuming and require extensive training and expertise. These requirements have limited the use of microscopy to clinics and hospitals in the urban areas of Ghana. In spite of these challenges, microscopy is still considered as the gold standard in the laboratory diagnosis of malaria.

The limitations of microscopy coupled with the relatively high endemicity of malaria in Ghana has led to the introduction of more simple methods such as the use of RDTs so as to minimize the incidence of presumptive diagnosis and treatment of malaria in Ghana. RDTs are simple to perform, do not require extensive equipment and expertise to perform and interpret the results [4]. The use of RDTs have simplified the diagnosis of malaria and also enhanced the proper prescription of antimalarial drugs with accompanying reduction in antimalarial drug resistance [5]. Highly malaria endemic nations that have fully implemented the use of RDTs in the diagnosis of malaria have significantly reduced the incidence of presumptive treatment of malaria. For example in Senegal records of 516,576 courses of inappropriate artemisinin-based combination therapy (ACT) prescription were averted after the introduction of universal parasite-based diagnosis using RDTs between 2007 and 2009 [6]. Similarly, a study conducted in Tanzania between 2006 and 2010 revealed that using RDTs reduced anti-malarial drug dispensing from 98.9% to 32.1% in children under 5 years [7].

Even though RDTs have simplified the diagnosis of malaria, WHO and other agencies such as Special Program for Research and Training in Tropical Disease (TDR), Foundation for Innovative New Diagnostics (FIND), and the Centre for Disease Control and Prevention (CDC) recommend that countries test the performance (sensitivity and specificity) of malaria RDTs before being approved. In 2011, Nkrumah and co-workers reported in a study conducted at Agogo Presbyterian Hospital in Ghana that Partec malaria RDT had sensitivity and specificity of 100% and 97.2% respectively while Binax Now malaria RDT had sensitivity and specificity of 97.4% and 93.6% respectively [8]. A similar study conducted at Kintampo Hospital in Ghana to evaluate the performance of CareStart™ RDT on 436 children using microscopy as the gold standard reported a sensitivity and specificity of 100% and 73% respectively [9]. Even though the reports from Nkrumah and co-workers as well as that of Baiden and co-workers suggested that the performance of Partec, Binax Now and CareStart™ RDTs were good inappropriate storage and transport conditions may affect the performance of these RDTs hence the need for regular surveillance to monitor the performance of RDTs that are used at regular interval. Using sub-standard or underperforming RDTs can lead to the generation of inaccurate results and this may lead to inappropriate management of malaria cases and therefore increase the risk of drug resistance as well as increased cost of treatment.

The aim of the current study was to evaluate the diagnostic accuracies of two commonly used malaria RDTs in Ghana. The test kits are First Response® (the brand procured by Ghana Health Service) and CareStart™ using microscopy as gold standard. Surveillance for the performance of RDTs in Ghana would provide clinicians and other health service providers the needed information which will enable them take accurate decisions in malaria diagnosis and management.

2. MATERIALS AND METHODS

2.1 Study Sites

The study was conducted at both the Komfo Anokye Teaching Hospital (KATH) and the Virus Research and Molecular Biology Laboratory of the School of Medical Sciences of the Kwame Nkrumah University of Science & Technology in Kumasi Ghana. KATH is the second largest tertiary hospital in Ghana which serves patients from the middle and northern parts of Ghana.

2.2 Sample Collection and Processing

The period of study spanned from October, 2014 to March, 2015. Four hundred (400) patients on admission at Paediatric Emergency Unit (PEU) at KATH with fever cases or clinically suspected cases of malaria were enrolled in the study. Patients who had been on oral anti-malarial drugs a week prior to admission or on intravenous ACTs within week prior to sample collection were excluded from the study. The

patients were assisted to fill one page structured questionnaires to provide information on their demographic data. Parts of blood samples which were collected from patients at PEU for routine laboratory diagnosis of diseases were used in the study. Since the patients were vulnerable it was not advisable to repeat invasive procedure to collect blood samples and so 5 ml of venous blood were collected from each patient at the PEU into ethylenediaminetetraacetic acid (EDTA) bottles under aseptic conditions. The samples were then transported to the haematology laboratory at the Accident and Emergency (A & E) unit of KATH where 50 µL were processed and analyzed for the study. The rest of the samples were used for routine laboratory diagnosis of diseases.

2.3 Microscopic Examination of Blood Samples

At the laboratory, the blood samples were well mixed and 6µl and 2µl were used to prepare thick and thin blood films respectively on the same slide. After air-drying the slides for about 45 minutes, the thin film portions were immersed in absolute methanol to fix. The slides were air-dried for 15 minutes in vertical positions with the thin film part below the thick film. The blood films were stained with freshly prepared 5% buffered Giemsa solution (pH 7.2) for 45 minutes after which the excess stained was washed off with buffered distilled water (pH of 7.2). After air-drying the slides in vertical positions, they were examined under X100 objective lens of the microscope by the two independent expert microscopists at the haematology laboratory at the A & E unit at KATH. Disagreement in results on the presence or absence of parasitaemia between the two experts microscopists were settled by referring to a third expert micrsocopist. The results of the microscopy and the RDT were not made available to any member of staff at the haematology laboratory at the A & E unit at KATH who read the slides until after the study. A blood film was considered negative when no malaria parasites or trophozoites were observed after 100 high power fields (hpf) had been examined on the thick film [10]. Where parasites were seen, they were counted against 200 white blood cells (WBCs). The parasite count per microliter (µl) of blood was obtained using the formula: (Parasite count/200WBC) × Absolute WBC count [11]. To ensure accurate parasite count for the thick films with high parasitaemia (≥100 parasites/high power field), parasites were counted in the thin film. In the thin film, parasitized RBCs were counted against 1000 RBCs. The parasite count per microliter (µl) was obtained using the formula: (parasitized RBCs/1000 RBCs) × Absolute RBC count [12]. Thin films were also examined for the type of species and stage of parasite.

2.4 Testing the Performance of RDTs

The malaria RDT kits used in the current study were First Response® (Premier Medical Corporation Ltd., India) and CareStart™ (Access Bio. Inc., U.S.A). These two RDTs make use of antibodies to detect *Plasmodium* antigen Histidine-rich protein 2 (HRP-2) which are produced as part of the developmental cycle in the human host.

The performance of First Response® and CareStart™ RDTs were evaluated following the manufacturers' instructions. For each rapid diagnostic test, the cassettes were first labelled with the sample number, then 5µl of thoroughly mixed whole blood was added to the sample well and the assay buffer completely emptied into the buffer well. The cassette was left for about 15 minutes to allow the capillary rise of the blood. The RDT reaction was considered as positive when two dark colour bands were seen at the control (C) and test (T) labels. The reaction was considered as negative when only one dark band was seen at the control (C) label. The reaction was considered as invalid when no bands were seen at both the control and test labels and or when a band was seen at the test label but not at the control label. All invalid reactions were repeated to determine results as either positive or negative.

2.5 Data Analysis

All data were entered and analysed using Microsoft excel 2013 software. The sensitivities and the specificities of the RDTs tested were calculated. The difference in sensitivities and specificities between the RDTs and microscopy were considered significant when p-value ≤ 0.05. Kappa values which expressed the strength of agreement between the gold standard and the RDTs were calculated with 95% confidence interval. A kappa-value of ≤ 0.60 was considered as moderate agreement. A kappa-value of > 0.6 < 0.8 was considered good agreement while a kappa-value > 0.8 was considered as a near perfect agreement [13].

3. RESULTS AND DISCUSSION

A total of 400 children comprising 161 (40.2%) females and 239 (59.8%) males on admission at the PEU with fever or with history suggestive of malaria were recruited into the study. The age of the study subjects ranged from 1 year to 17 years with mean age of 4.8 years (SD ± 3.9 years).

Upon microscopic examination of the blood films by two independent expert microscopists, *Plasmodium* parasites were seen in 33 (8.3%) of the blood films. The level of agreement between the two independent microscopists was negligible or statistically the level of agreement between the two microscopists was 0.91. Of the 33 positive blood films, 30 (90.9%) were positive for *Plasmodium falciparum* mono-infection, 1(3.1%) was positive for *Plasmodium malariae* mono-infection while 2 (6.0%) were positive for *P. falciparum* and *P. malariae* mixed infections. Most of the Plasmodia seen were at the trophozoites stage (97.0%) while extremely few of them had gametocytes on the trophozoite (3.0%).

The First Response® malaria RDT revealed that 65 of the blood samples were positive for malaria with 32 of them being in concordance with microscopy which was considered as the gold standard indicative of the fact that 33 of the results were false positive. Similarly, the CareStart™ malaria RDT also revealed that 68 of the blood samples were positive for malaria with 32 of them being in concordance with microscopy suggestive of the fact that 36 of the results were false positive Table 1. Table 2 describes the performance characteristics of First Response® and CareStart compared to microscopy. The sensitivities of the First Response® and the CareStart™ malaria RDTs when compared with microscopy the gold standard were 97.0% (95% CI: 84.2-99.9) and 97.0% (95% CI: 84.2-99.9) respectively. The specificities were as follows: First Response® RDT 91.0% (95% CI: 87.6-93.7) and CareStart™ RDT 90.2% (95% CI: 86.7-93.0) as shown in Table 2. The strength of agreement (kappa) between microscopy and the two RDTs with 95% confidence interval was good and moderate for the First Response® and the CareStart™ RDTs respectively (Table 2). The PPV index was higher for First Response® (49.2; CI 36.6-61.9) reflected by less false positives compared to CareStart™ (47.1; CI 34.8-59.6). However, the NPV was the same for the two RDTs.

The current study evaluated the performance or diagnostic accuracy of First Response® and CareStart™ RDTs in the diagnosis of *Plasmodium falciparum* induced malaria at KATH in Kumasi, Ghana using the traditional giemsa stain microscopy as the gold standard. The WHO recommends that for an RDT to qualify to be used in the diagnosis of malaria, it must have at least 95% sensitivity and specificity of at least 90% [14]. The sensitivity and the specificities of the two RDTs reported in this study were good indicating that the First Response® and the CareStart™ RDTs could be relied upon in accurate diagnosis of suspected malaria cases in Ghana. The 97% sensitivity of CareStart™ RDT reported in this study was similar to that reported by Baiden and co-workers in a study conducted at the Kintampo Hospital in the Brong Ahafo Region of Ghana [9]. However, the specificity (90.2%) reported in this study was relatively higher than that (73%) reported by Baiden and co-workers. The difference in specificities could be attributed to different batches of the CareStart™ RDT that were used in the different studies.

Table 1. Results of each RDT in comparison with microscopy (Gold Standard)

	RDTs		Microscopy
	First response	Care start	
Positive	65	68	33
Negative	335	332	367
Total	400	400	400

Table 2. Accuracy indices of the RDTs compared to microscopy to detect HRP-2 of *P. falciparum*

Test methods	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Kappa (95% CI)	SE of kappa	DP (95% CI)
First response	97.0 (84.2-99.9)	91.0 (87.6-93.7)	49.2 (36.6-61.9)	99.7 (98.4-99.9)	0.61 (0.50-0.73)	0.06	8.3 (5.8-11.4)
CareStart	97.0 (84.2-99.9)	90.2 (86.7-93.0)	47.1 (34.8-59.6)	99.7 (98.3-100)	0.59 (0.48-0.71)	0.06	8.3 (5.8-11.4)

PPV: Positive predictive value; NPV: Negative predictive value; DP: Detection prevalence

Both the First Response® and CareStart™ RDTs exhibited 1 false negative result each. It is a well-known fact that the First Response® and CareStart™ malaria test kits are HRP-2-based that rely on malaria monoclonal antibodies in detecting HRP-2 antigen in human blood samples. The HRP-2 antigen is normally produced by the young gametocytes and the asexual stages of *P. falciparum* [15]. Because of the relative abundance of HRP-2 in *P. falciparum*, it was the main antigen used to develop RDTs for the detection of *P. falciparum*. The 1 false negative result reported could be that the RDTs employed in the current study could not detect the *P. malariae* which lacks the HRP-2 antigen but may have other antigens such as plasmodium lactate dehydrogenase (pLDH) and plasmodium aldolase (pAldo) [4].

The results from the study indicated that 33 of the results were false positive for First Response® RDT while 36 were false positive for the CareStart™ RDT. The false positive malaria RDT results could be caused by antigen's persistence 28 days after treatment [4,16] and the fact that the HRP-2 antigens are produced by the schizonts at early stage of the parasite even before the parasite are initially released into peripheral circulation.

The limitation to the current study are our inability to use RDTs which will detect both HRP-2 and pLDH such as First Response Malaria pLDH/HRP-2 combo® (Premier Medical Corporation Ltd, India, Catalogue No: l16FRC30) and CareStart Malaria pLDH/HRP2 combo™ (Access Bio Inc.,NJ, USA, Catalogue No: G0131), two of the best performing RDTs indicated in the WHO/FIND round 1–3 report [17] and our inability to use PCR as the gold standard to compare the detection accuracy of the two RDTs. It is recommended that, malaria RDTs should be used only as first line of diagnosis while clinicians await results from microscopy. RDTs should be used often in remote areas where microscopy is a challenge. Procurement of malaria RDTs into the country should be preceded by RDT accuracy testing within the country by Ghana Health Service. These measures if employed would improve malaria diagnosis and management in Ghana.

4. CONCLUSION

The First Response® and CareStart™ which were the commonest malaria RDTs used in Ghana at the time of the study have good detection accuracy and compare favourably to WHO's RDT standards and are capable of accurately diagnosing malaria.

CONSENT

The authors declare that informed consent was obtained from each patient or the care taker after the purpose of the study had been explained to them in a language that they understood.

ETHICAL APPROVAL

The authors declare that all experiments were examined and approved by the Committee on Human Research Publication and Ethics (CHRPE) of School of Medical Sciences of the Kwame Nkrumah University of Science and Technology (KNUST) and KATH in Kumasi, Ghana.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. WHO. World Malaria Report. December 2015 Fact Sheet; 2016.
2. Crump JA, Morrissey AB, Nicholson, WL, Massung, RF, Stoddard, RA, Galloway RL, et al. Etiology of severe non-malaria febrile illness in Northern Tanzania: A prospective cohort study. PLoS Negl Trop Dis. 2013; 18:7(7):e2324.
3. Malm KBPC, Armah G, Gyapong J, Owusu-Adjei S, Koram K, Binka F. Malaria as a cause of acute febrile illness in an urban paediatric population in Ghana. Proceedings of the Annual Meeting of the American Society of Tropical Medicine and Hygiene, Atlanta, GA, November 11–15, 2012.
4. Moody A. Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev. 2002;15:66–78.
5. Chandler CIR, Whitty CJM, Ansah EK. How can malaria rapid diagnostic tests achieve their potential ? A qualitative study of a trial at health facilities in Ghana. Malaria Journal. 2010;9(95):1–15.
6. Diouf ML, Birame M, Thiam S, Thior M, Faye B, Diallo I, et al. Major reduction in anti-malarial drug consumption in Senegal after nation-wide introduction of malaria

rapid diagnostic tests. PLos ONE. 2011; 6(4):1-6.
7. Ishengoma DS, Francis F, Mmbando BP, Lusingu JPA, Magistrado P, Alifrangis M, et al. Accuracy of malaria rapid diagnostic tests in community studies and their impact on treatment of malaria in an area with declining malaria burden in north-eastern Tanzania. Malaria Journal. 2011;10(1): 176.
8. Nkrumah B, Acquah SEK, Ibrahim L, May J, Brattig N, Tannich E, et al. Comparative evaluation of two rapid field tests for malaria diagnosis : Partec rapid malaria test ® and binax now ® malaria rapid diagnostic test. BMC Infectious Diseases. 2011;11(1):143.
9. Baiden F, Webster J, Tivura M, Delimini R, Berko Y, Amenga-Schandramohan D. Accuracy of rapid tests for malaria and treatment outcomes for malaria and non-malaria cases among under-five children in rural Ghana. PLosONE. 2012;7(4):1–9.
10. Cheesbrough M. District laboratory practice in tropical countries. Cambridge; New York: Cambridge University Press, 2; 2005.
11. Jeremiah ZA, Uko EK. Comparative analysis of malaria parasite density using actual and assumed white blood cell counts. Annals of Tropical Paediatrics. 2007;27(1):75-79.
12. Frean JA. Reliable enumeration of malaria parasites in thick blood films using digital image analysis. Malaria Journal. 2009; 8(1):218.
13. Altman DG. Practical statistics for medical research. Boca Raton London New York Washington, D.C: Chapman and Hall, 1; 1991.
14. WHO. Good practices for selecting and procuring rapid diagnostic tests for malaria. WHO Global Malaria Programme. Geneva, Switzerland. 2011;15.
15. Rock EP, Marsh K, Saul SJ, Wellems TE, Taylor DW, Maloy WL, et al. Comparative analysis of the *Plasmodium falciparum* histidine-rich proteins HRP1, HRP2 and HRP3 in malaria diagnosis of diverse origin. Parasitology. 1987;95:209–227.
16. Humar A, Ohrt C, Harrington MA, Pillai D, Kain KC. Parasight F test compared with the polymerase chain reaction and microscopy for the diagnosis of *Plasmodium falciparum* malaria in travellers. Am J Trop Med Hyg. 1997;56: 44–48.
17. FIND/WHO. Malaria rapid diagnostic test performance: Results of WHO product testing of malaria RDTs: Round 3 (2010–2011). Geneva: World Health Organization; 2010.

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