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Research Article

Performance of Advanced Quality One Step ™Malaria (P.F.) for Rapid Diagnosis of Symptomatic *Plasmodium Falciparum* Infections in Tanzania

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Abstract

Background: The use of rapid diagnostic tests (RDTs) represent an alternative to microscopy for malaria diagnosis by providing parasite-based diagnosis in areas where good quality microscopy cannot be maintained. This study evaluated the diagnostic performance of the commercial Advanced Quality One Step ™ Malaria (p.f.) test compared with light microscopy for the diagnosis of symptomatic *Plasmodium falciparum* malaria at primary health care level in Tanzania.

Methods: Capillary blood samples were collected from 613 febrile patients attending the Kongo primary health facility, Bagamoyo District, Tanzania, from March-June 2014. The blood samples were prepared and examined immediately by microscopy of Giemsa stained thick blood smears and the Advanced Quality One Step ™ Malaria (p.f.) test. Light microscopy was used as Gold standard for the diagnostic performance of the RDT at test. Statistical analysis was performed using Stata version 11.

Results: Overall 39.5% (242/613) *P. falciparum* infections were detected by microscopy compared with 38.8% (238/613) by the RDT. The sensitivity and specificity of the RDT was 95.9% (95% CI 92.5-98.0%) and 98.4% (95% CI 96.5-99.4%) respectively, with corresponding positive and negative predictive values of 97.5% (95% CI 94.6-99.1%) and 97.3% (95% CI 95.2-98.7%)

Conclusion: The Advanced Quality One Step™ Malaria (p.f.) test provided a high diagnostic accuracy for detection of symptomatic *P. falciparum* infections, comparable with gold standard light microscopy. This indicates that the RDT could be considered for the use in primary health care facilities in Tanzania where high quality light microscopy is not available.

Keywords: Advanced Quality One Step™; Light microscopy; Malaria; Rapid diagnostic test; Tanzania

Introduction

An estimated 627,000 malaria deaths occurred in 2012, mostly in African children and ma ny of them preventable with prompt diagnosis and treatment [1]. The main control measures in endemic countries rely on early and prompt diagnosis and treatment together with vector control to reduce transmission. It is not possible to accurately diagnose malaria using clinical algorithms alone, as the signs and symptoms of malaria, e.g. fever, chills, headache and anorexia, are nonspecific and thus common to many other febrile diseases and conditions [2,3]. Improved diagnosis before prescribing anti malarial treatment will improve treatment outcomes, rationalize health care costs by reducing drug consumption and minimize drug pressure and thus the risk of resistance development, and assist surveillance of malaria infection [2-4]. The revised World Health Organization (WHO) guidelines recommend that malaria case management should be based on parasite-based diagnosis by microscopy or antigen detecting rapid diagnostic tests (RDTs) in all cases [2]. However, in many endemic African countries prompt, accurate results from microscopy cannot be delivered efficiently, and access to diagnosis is limited, an estimated over 80% of malaria treatments are applied without diagnostic testing [1].

The use of RDTs represents an alternative to microscopy for malaria diagnosis by providing parasite-based diagnosis in areas where high quality microscopy cannot be maintained. RDTs are immunochromatographic tests that detect parasite-specific antigens in a finger-prick blood sample [5]. Some tests detect only one species (*P. falciparum*), others detect one or more of the other species of human malaria parasites (*P. vivax, P. malariae and P. ovale*) [6-8]. The WHO also recommends that RDTs be implemented with a comprehensive quality control strategy, that include purchase from a manufacturers that follow good manufacturing practices (GMP) and each lot of RDTs should be tested on arrival in the country of use [5,9].

To be widely useful, a RDT must have high sensitivity of > 95% in detecting plasmodia at densities of more than 100 parasites per µl of blood, to ensure all clinically-significant malaria infections are detected [10]. RDTs should also have high specificity for appropriate management of non-malarial fever and high stability to allow

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transport and storage in ambient conditions in malaria-endemic areas. [11]

The commercial Advanced Quality One Step™ Malaria (p.f.) (Intec products, INC, XIAMEN, P.R. China) test, is a new RDT introduced in Tanzania for diagnosis of malaria after successful laboratory-based evaluation [12]. The test is an antigen-capture assay detecting presence of a specific soluble protein, histidine-rich protein II (HRPII) which is present in and released from, *P. falciparum* infected red blood cells. The assay is intended for use with whole blood and does not require additional instruments. A capture monoclonal antibody is immobilized on the nitrocellulose strip. The red blood cells are lysed releasing HRP-II which, binds selectively to this antibody as the blood is wicked up the strip. The RDT had previously not undergone any field evaluation in Tanzania.

This aim of this study was to evaluate the diagnostic performance of Advanced Quality One Step Malaria (p.f.) test, for the diagnosis of clinical *P. falciparum* malaria relative to thick blood smear microscopy at one primary health care facility in Bagamoyo District, Tanzania.

Materials and Methods

Study site and area

This was a health facility based study conducted in Bagamoyo District among acute febrile patients attending the Kongo primary health facility from March-June 2014. The district has a peak malaria transmission from April-June and to a lesser extent in November to December. The district is still malaria endemic despite scale up of interventions to control malaria. The district has a population of more than 200,000 and the majority of its population depends on subsistence farming. Malaria diagnosis based on light microscopy and treatment are available free of charge in the study site.

Patients and study team

Acute febrile patients of all ages attending the out-patient clinic at Kongo dispensary during the study period were screened for eligibility. Eligible patients were those with a body temperature > 37.5°C or history of fever during the last 3 days. Individuals who reported intake of anti malarial drugs within the last 2 weeks before enrolment or refused consent were excluded. Advanced Quality One Step™ Malaria (p.f.) Test and thick smear microscopic examination was performed on each of the acute febrile patients.

The study team consisted of three health workers employed at the health facility. They received a one day pre-study training on how assess and select patients according to study protocol, collection of blood samples for laboratory diagnosis, and use Advanced Quality One Step Malaria (p.f.) test, according to the manufacturer's instructions and how to fill case record forms.

Data collection and processing

Patients' age, presenting symptoms and history of anti malarial treatment in the past two weeks before enrolment were recorded using a case record forms. Finger-prick blood samples were collected and placed in a grease-free, clean, glass slide. The same finger-prick blood sample was used to carry out the RDT in parallel, following manufacturer's instructions. Thick blood smears were prepared, and slides were stained in 10% Giemsa solution for 10 minutes. Slide reading was done at the health center by an experienced laboratory technician and the result was considered negative if no parasites were seen after examination of 200 fields at 1,000x magnification. The technician was blinded to the results of the RDTs. All blood smears were re-read by an experienced microscopist at the Department of Parasitology Laboratory, Muhimbili University of Health and Allied Sciences (MUHAS) blinded to the initial microscopy and RDT results. In case of discordant between the two readers, a third expert reader was used. The results of the third expert reader were considered decisive.

The Advanced Quality One Step™ Malaria (p.f.) (Intec products, INC, XIAMEN, and P.R. China) used in the study was supplied by the manufacturer to the Ministry of Health for quality testing prior to registration. The quality of package was checked before use. Test kits were stored at room temperature and used for malaria diagnosis according to the manufacturer's instructions. One drop (10µl) of fresh blood was collected and transferred to the sample pad ("S" well) of the test card using the plastic dropper provided the manufacturer. All RDTs were labeled with patients' identification numbers and results were recorded within 15minutes after adding three drops of a clearing buffer. The presence of a unique HRP-II, black line (test line) was interpreted as an infection with *P. falciparum*. The presence of an upper black line (the procedural control line) demonstrates the test has been performed correctly. A test result without a control line was considered invalid. Invalid tests were retested by taking fresh blood from each patient who had an invalid test result.

Data entry and analysis

Data were double entered and validated by Epi Info™ 6. Analysis was performed with STATA version 11 (Stata- Corp, College Station, Texas). Data are presented as frequencies and proportions with corresponding 95% confidence intervals (CIs). The Stata command diagti was used to calculate sensitivity, specificity and positive and negative predictive values using thick blood smear microscopy as the gold standard.

Ethical clearance

The study was reviewed and approved by the Ethical Committee of Muhimbili University of Health and Allied Sciences. All patients provided written informed consent before study enrolment. Malaria positive cases were treated with anti malarial drugs based on the current national treatment guidelines of Tanzania.

Results

Baseline characteristics of patients

A total of 613 acute febrile individuals were examined for malaria parasites by the Advanced Quality One Step Malaria (p.f.) tests and thick blood smear microscopy at Kongo dispensary. The male: female ratio was 1:1.1. The median age was 5 years (range 1-72 years). All patients had history of fever in the past two days or raised temperature ≥37.5°C, and 102/613(16.6%) reported history of vomiting during the current illness.

Thick blood smear microscopy detected *P. falciparum* infections in 39.5% (242/613) of patients. The geometric mean asexual parasite density was 5216µl (range 120-200,000/µl) (Table 1). The parasite density distribution showed that about 3% of cases had <100 parasites/µl, whereas 17.4% (42/242) had parasite densities 100-1000/

µl and 77.8% (193/242) >1,000 parasites/µl. Conversely, Advanced Quality One Step Malaria (p.f.) were positive in 38.8% (238/613) of the tested individuals (Table 2).

Diagnostic performance of Advanced Quality One Step™ Malaria (p.f.)

The sensitivity and specificity of the RDT were 95.9% (95% CI 92.5-98.0%) and 98.4% (95% CI 96.5-99.4%), respectively, compared with light microscopy (Table 2).The corresponding positive and negative predictive values were 97.5% (95% CI 94.6-99.1%) and 97.3% (95% CI 95.2-98.7%) There were 16/613 (2.6%) discordant RDT and thick blood film results, of which six RDTs had faint bands and were negative by microscopy and 10 were negative by RDT but positive by microscopy (Table 2). The parasite densities for patients with negative RDT results ranged from 80-200 p/µl. There was no reported invalid RDT result.

Discussion

The use of a parasite based confirmatory diagnosis with either microscopy or RDTs is expected to reduce the overuse of anti malarial drugs by ensuring that treatment is targeted to the patients with confirmed malaria infection, as opposed to treating all patients with fever as malaria. To our knowledge this is the first study to report afield evaluation of the diagnostic performance of Advanced Quality One Step™ Malaria (p.f.) for clinical *P. falciparum* malaria in Tanzania. The RDT revealed high sensitivity and specificity of >95% when compared with the gold standard microscopy. This is in agreement with other commercially available HRP-II based RDTs, even though there are reports of substantial variability in their field performance with sensitivities of the tests ranged from 42% to 100%, specificities from 65% to 100% [6,7,11]. The accuracy of RDTs in detecting malaria infections might be affected by various factors including manufacturing process as well as environmental conditions, concentration of the target antigen in the host blood, and the accuracy of the reference standard [3,11], why a robust quality control system is critical to ensure high diagnostic accuracy of RDTs in the field.

Overall, we observed ten false negative results by Advanced Quality One Step™ Malaria (p.f). This is similar with other commercially available HRP-II based RDTs, with reported false negative results in about 5% of *P. falciparum* cases [7]. Importantly, all 10 negative RDTs in our study had low parasite densities as assessed by microscopy, i.e. <200µl, which is close to the detection limit of most commercially available RDTs. Thus no high density parasitemia was missed by the RDT.

Table 1: General Characteristics of Patients.

Total		613
Median age (years) range		5 (1-72)
Sex (%)	Female	344 (56.1%)
Geometric mean parasite density (range)/µl		5216 (120-200000)
Parasite density distribution* (n=242)	<100/µl	7 2.9%
	100 - 1,000/µl	42 17.4%
	>1,000/µl	193 77.8%

***** Samples with positive results

Table 2: Advanced quality one step™ Malaria (p.f.) Test sensitivity, specificity and predictive values.

	Microscopy		
ADVANCED QUALITY ONE STEP™ Malaria (p.f.) Test	Positive	Negative	
Positive	232	6	
Negative	10	365	
Sensitivity (95% CI)I%	95.9%	92.5%	98.0%
Specificity (95% CI)%	98.4%	96.5%	99.4%
Positive Predictive Value (95% CI)%	97.5%	94.6%	99.1%
Negative Predictive Value (95% CI)%	97.3%	95.2%	98.7%

In addition, Advanced Quality One Step™ Malaria (p.f.) showed false positive results in six cases, characterized by faint bands. It is well known that HRP-II based RDTs can remain positive several weeks after successful treatment for *P. falciparum* malaria [13], and thus give rise to false positive results. Other causes of false positive results include presence of sole gametocytaemia; cross-reactions with some assays have been reported for patients with rheumatoid factor or other circulating auto-antibodies and poor slide preparation or reading [3,6,11,14]. Taking history of recent anti malarial intake and excluding those with report recent use probably reduced the number with false positive RDT results in our study.

Challenges in assessing RDTs performance include use of a reference standard (microscopy), which is highly dependent on the performance of technicians, transport and storage of RDTs [3,11]. Other limitations in the field include end-user's errors, procedure (delayed reading, incorrect sample and buffer volumes) and interpretation (not recognizing invalid test results, disregarding faint test line) [3]. This study mitigated these challenges by employing experienced technicians, and training health workers on use of RDTs according to manufacturer's instructions. Blinding of RDTs and microscopy results reduced likelihood of bias. The RDTs were transported and stored at the study site according to specified storage recommendations around between 28°C and 30°C, and by using fresh samples from patients, followed all manufacturers' instructions.

We used microscopy as gold standard for the diagnostic evaluation of the RDT. However, the results may have changed if molecular diagnostics such Polymerase Chain Reaction (PCR) [7,15] had been used instead as reference standard, since PCR is highly sensitive diagnostic tool at low levels of parasitemia [16]. However, PCR is also subject to various technical limitations such as false positive results due to contamination of samples if laboratory standards are not sufficiently high. Moreover, PCR is expensive and require skilled staff hence not widely available in many malaria endemic countries and has long-time-to result. Thus, PCR may presently not be an appropriate alternative to microscopy as gold standard for field evaluation of malaria diagnostic tools in resource limited settings [3]. Still, previous studies have shown that PCR reduced false positive results in areas with low malaria prevalence and increased estimates of specificity/sensitivity compared to microscopy [7]. Other diagnostic techs on horizon which will improve assessment RDTs include Loop Mediated Isothermal Amplification (LAMP) a highly sensitive diagnostic tools at very low levels of parasitemia [17].

Conclusion

The Advanced Quality One Step™ Malaria (p.f.) test provided a

high diagnostic accuracy for detection of symptomatic *P. falciparum* infections, comparable with gold standard light microscopy. This indicates that the RDT could be considered for the use in primary health care facilities in Tanzania where high quality light microscopy is not available.

Authors' Contribution

BN was involved in the study conception and design, data analysis, and drafting the manuscript. BN, SNB and UHN were involved in data collection and reviewing the manuscript. All the authors have read, edited and approved the manuscript.

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