Efficiency of Urine Malaria Test Kit for the Diagnosis of Malaria of Febrile Patients in Gombe, Nigeria

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Abstract

The diagnostic efficiencies of urine malaria test kit with microscopy as the gold standard in detecting Plasmodium falciparum HRP-2, a poly-histidine antigen in urine of febrile patients was determined. The study was conducted in a primary and secondary health institutions in Gombe town. Participants were enrolled based on presentation with fever (≥37.5°C). Blood samples were collected by phlebotomy. Thick and thin blood films were prepared, stained and examined for malaria parasite, under the microscope using the oil immersion objective for thick films. Meanwhile, urine samples were collected in urine sample bottles, labeled and immediately tested using specially designed urine Rapid Diagnostic Test (RDTs).

With the blood smear microscopy as standard, the disease prevalence was 67.3% and sensitivity for the UMT was 73.4% respectively. The Urine malaria test kits (UMT) had a specificity of 90.7% as well as positive and negative predictive values of 93.5%and 55.6% respectively.

The rapid diagnostic kits showed moderate level of sensitivity compared with blood smear microscopy as it showed considerable acceptable levels in the diagnosis of malaria.

Keywords: Diagnostic kit, *Plasmodium falciparum*, Prevalence, Microscopy, Urine.

1. INTRODUCTION

Malaria is the most important insect transmitted disease Gilles and Warrell (k1993) and is considered one of the most important of all tropical diseases in terms of morbidity and mortality on a global scale (Taure and Oduola 2004). It is estimated that some 2 billion individuals are at

risk with over 100milloin developing overt clinical diseases and more than 1.5 – 2.7 million deaths every year (Taure and Oduola 2004). Malaria is a complex disease that varies widely in epidemiology and clinical manifestations in different parts of the world. Variable factors such as distribution and efficiency of mosquito vector, climate and other environmental conditions and the behaviour and level of acquired immunity of the exposed human population contributes to wide distribution of malaria. The disease is mainly responsible for over 25% infant mortality, 30% childhood deaths and 11% maternal deaths resulting in an economic burden within the country to be at over 13 billion Naira(₦) in loss annually in terms of treatment, prevention cost and loss of man hours (FMH, 2005 and 2009a).

Malaria is one of the most common infectious disease and an enormous public health problem caused by protozoan parasites *Plasmodium*, with four species known to infect humans and the most serious form of the disease caused by *P. falciparum* and *P.vivax.* The species *P. falciparum* was reported to be the most predorminant cause of malaria in Nigeria (Erhabor *et al* 2006). An estimated 85% of cases and 90% of carriers (mainly asymptomatic) are found in tropical Africa. Twenty to Thirty (20 – 30%) deaths in children have been attributed to the disease (Greenwood *et al* 1986; Oguonu and Okafor 2007).

It has been noted that most malaria deaths occur at home hence limited reported cases or not reported (Rugemalila *et al* 2006). Children and pregnant women are at the greatest risk, due to lack of adequate immune protection against the disease (WHO 1996 and Schwartlander 1997). In areas where *Plasmodium falciparum* is endemic, delay in treatment of uncomplicated malaria may result in severe disease in these most vulnerable groups (WHO/OMS, 2002).

Early diagnosis and treatment are therefore crucial in preventing complicated malaria as it is the most dangerous form of disease with high levels of the parasite in blood (parasitaemia) alone resulting in deaths or produce fatal cerebral , renal or pulmonary complications particularly in non-immune individuals. Diagnosis of Malaria has been the most neglected area of malaria research as such accurate diagnosis is the only way of effecting rational therapy with confirmation of diagnosis to help aid in malaria control today. With the enormous burden and public health significance of malaria, various approaches for its control have been put in place chiefly among these approaches is chemotherapy, but its success have not been fully felt because of its reliance on effective and early diagnosis of the causative agent. Among several diagnostic

protocols that are currently available, microscopy is currently the gold standard but it has its draw backs. The need for continual search for alternative diagnostic techniques that are acceptable and efficient as microscopy is more desired.

The development of a simple, accurate, fast and non-invasive rapid urine-based dipstick assay for diagnosis of malaria has led to timely diagnosis and treatment of the disease, as such eliminating the complications and death that could result from delayed treatment. Home-based management of malaria (HBMM) which is recognized as an important strategy for reducing the morbidity and mortality of the disease, thrives on accurate diagnosis outside the health facility, usually in the home by parents, caregivers and/ or by oneself. Home based management of malaria (HBMM) is, presently done using the presumptuous symptomatic diagnostic approach, because of lack of technical skills and logistics such as microscopes (Oguonu and Okafor 2007).

It is in a bid to improve upon the limitations of the current diagnostic tools this study proposes to study the effectiveness of rapid urine-based field-applicable dipstick test for diagnosis of malaria in infected individuals using *Plasmodium* antigens excreted in the urine of infected subjects, so as to compliment home based management of malaria.

2. MATERIALS AND METHODS

2.1 Sample Collection

Two diagnostic tests were used in the study which includes microscopy and Urine Malaria Test Kit.

2.2 Urine Sample. Urine samples were collected from study patients in universal bottles, marked and immediately tested in accordance with W.H.O standards (WHO, 2010).

2.3 Blood Sample. Each patient after successful enrollment for the study are either finger pricked or blood were drawn with syringes via venipuncture using 5ml sterile disposable needle where about 2 ml of blood was collected from each subject by the attending phlebotomist. The upper cubic forsa of the upper arm was first sterilized with 70% alcohol using cotton wool and using a tunicate tied to the upper cubic-forsa of the left arm, a 5ml syringe was used to draw 2ml of venous blood which was placed in vacutainers containing EDTA. These were then packed in racks after proper labelling and transported to the laboratory in compliance with all ethical

regulations with careful attention to technique used during blood collection and handling of samples WHO, (2010) for microscopic analysis.

3. TESTING METHODS.

3.1 Urine Sample testing principle.

Testing is carried out when a test strip with UMT Kit (Lot No.7014978, Fyodor Biotechnologies, Baltimore Maryland, USA) is dropped into a clean container containing up to 100 µl of urine. The strip is allowed to wick saturating the strip for 1-2 minutes and is allowed to stay at room temperature for 20-25 minutes. The results were reported as negative, positive, or un-interpretable: if two visible lines appeared on the strip (even if very faint) the test was positive; if only the control line appeared, the test was negative. Tests results reported as un-interpretable, i.e., failure to observe a control line or the presence of a darkly stained background that obscured the test lines, were repeated to resolve the discrepant event. All safety precaution were followed according to manufacturer's instruction on the kit insert.

Red or purple band appears in both the control window as well as in the test line gives a positive test while only a red or purple color band on the control line gives a negative test. No color appearance in both the control line and test line gives an invalid test.

3.2 Blood Film

The blood samples drawn from study subjects were used for microscopic diagnoses of malaria parasite (WHO, 2010). Theses blood samples obtained were used to prepare thin and thick films on a grease free slide for testing. Two blood films were prepared for each subject, a 12µl of blood was spread over a diameter of 15mm for a thick blood film while a 2µl of blood was used for the thin blood film and using another clean slide to spread the blood drop to get a thin film by placing at the edge at an angle of 45^0 on the blood drop and pulled forward to make an even spread with the tail end.

3.3 Thin Film.

Using a clean slide as a spreader, with the slide containing a drop of 2µl of blood placed on a flat firm surface labelled with patients identity, by touching the slide with the spreader allowing the blood to run along its edge maintain an even contact with the blood sample at an angle of 45% and not extending to the edges to avoid autoinfection. This was allowed to air dry and then fix in absolute methanol for 2 seconds to prevent lysis of the red blood cells. It was

allowed to air dry on the rack and stained with Leishman's stain for 2 minutes (Chessbourgh and Prescot, 1987; WHO, 2010)

3.4 Thick Film.

After spreading the 12µl of blood on a clean slide and allowed to dry labelled with patients identity, the films were stained with 3% Giemsa prepared at pH 7.2, allowed to stand dry for 45 min then rinsed in clean water on the slide racks to drain dry before laboratory reading of slides were taken in accordance with W.H.O standard microscopy technique (Chessbourgh and Prescot, 1987; WHO, 2010).

3.5 Examination of blood films.

Thin blood film were examined to confirm for the presence of *plasmodium* species in parasitized cells. A slide is defined as negative if no parasites were seen after examining 100 fields but if the slide showed a positive result, the microscopists counted and recorded the asexual parasite counts against 200 white cells or 500 WBC with the number of asexual forms and sexual forms present recorded separately.

Thick film were examined by placing a drop of immersion oil unto the blood film covering a diameter of 10mm and scanning of the blood film is carried out using the X100 objective to examine for malaria parasite.

A definitive diagnosis of malaria from blood films was made when a reddish chromatin dot with a purple to blue cytoplasm of the malaria parasites are seen together under the microscope. The slides are further adjusted to observe the thin film for parasite identification, concentration and clarity.

4. RESULTS

The following variables were determined using microscopy as gold standard: true positive (TP), false positive (FP), true negative (TN) and false negative (FN). Test performance indices like sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false positive rate (FPR), false negative rate (FNR) and efficiency (J-Index) were also calculated. The sensitivity of the RDT (Urine Malaria Test kit) was also determined in relation to parasite densities. Table 1 shows study participants with a total of 199 subjects in the analysis where the incidence rates were 24.1% by UMT, 32.6% by microscopy at Federal Teaching Hospital (FTH)

respectively. It also showed an incidence rate at Specialist Hospital (SH) of 22.6% for UMT and 34.6% with microscopy. All the participants presented with fever >37.5^{0}C at enrollment with a median body temperature of 38.8^{0}C, (mid quartile range: 37.5^{0}C to 42.2^{0}C).the result shows a total incidence rate for UMT at 46.7% and 67.3% for microscopy.

Table 1: Incidence of malaria diagnosis at both FTH and SH Gombe. (June-December)

Technique / Location	UMT		Microscopy	
	No examined	**No (%) Infected**	**No examined**	**No (%) Infected**
FTH	99	48 (24.1)	99	65 (32.7)
SH	100	45 (22.6)	100	69 (34.6)
Total	**199**	**93 (46.7)**	**199**	**134 (67.3)**

Table 2 shows the sensitivity of urine malaria test kit as compared with microscopy in relation to parasitaemia levels which shows that the UMT was unable to detect malaria parasite (HRP-2) below 100 parasite/ µl as it has its limit of detection at 125 parasite/µl. At higher parasitaemia, the UMT was able to detect parasite antigen at 80.3% to 100% at level ≤200 and >201 parasite/µl.

Table 2: Sensitivity of diagnostic method as compared to Gold standard according to parasitaemia levels.

Parasitaemia level (parasite/µl)	No. Positive by microscopy (n=134)	No. Positive by UMT (n=93)	Sensitivity of UMT	Sensitivity of Microscopy	
≤100 (+)	21	0	0	100	
≤200 (++)	102	82	80.3	100	$P < 0.05$
≥201 (+++)	8	8	100	100	
≥500 (++++)	3	3	100	100	

Table 3:

Comparism of UMT against Giemsa stained microscopy for malaria parasites Diagnosis.

UMT	Microscopy		Total
	Positive	**Negative**	
Positive	87	6	**93**
Negative	47	59	**106**
Total	**134**	**65**	**199**

$P < 0.05$

It can be seen on Table 3, that the true positive test was 87, false negative test was 47, false positive test was 6 and true negative test was 59. The total positive result gave 134 and 93 for microscopy and UMT respectively. The sensitivity of UMT was 65% and specificity of 90.7% as the positive predictive value was 93.5%, negative predictive value 55.6% with a false negative rate at 35%, false positive rate at 9.2% as shown on Table 4.

Table 4: Diagnostic evaluation of Urine Malaria Test Kit (UMT).

Performance characteristics	UMT
Sensitivity	65.0%
Specificity	90.7%
Positive Predictive Value	93.5%
Negative Predictive Value	55.6%
False Negative Rate	35.0%
False Positive Rate	9.2%
Efficiency (Accuracy)	0.7336 (73.36%)

$P < 0.05$ (kappa= 0.467)

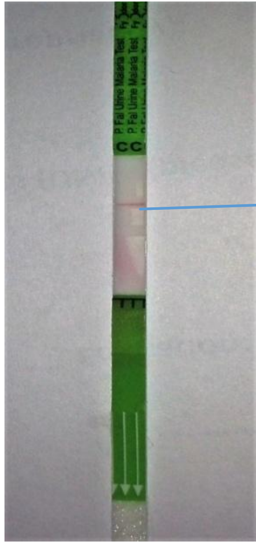
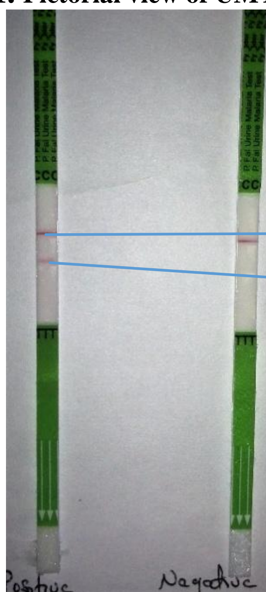


Plate 1. Pictorial view of UMT test showing negative result.



Control line (C)

Test Line (T)

Plate 2. Schematic representation of UMT test showing both positive and negative result.

5. DISCUSSION

The effectiveness of urine Malaria test kit from this study showed that it had an overall sensitivity of 65.0% and specificity of 90.7% in diagnosis of malaria among febrile patients attending the general outpatient departments of both Federal Teaching Hospital Gombe and the State Specialist Hospital Gombe. The study showed the capability of the test kit strip in detecting

the antigen of *Plasmodium falciparum* (HPR-2) in the urine of febrile patients at reliable levels of 73.36% (kappa 0.467). This result compares favorably with that of previous studies by Oguonu *et al* (2014) however reported a variable sensitivity of UMT of 83.75% and specificity of 83.48%, theses however varied with this study in sensitivity and specificity as the study gave a 73.4% slightly lower in sensitivity but a higher specificity than previous reports on the use of UMT as a diagnostic test for malaria (Oguonu *et al.,* 2014) during a pivot testing in South-east Nigeria. The UMTs method as compared to microscopy is easier to perform and samples can easily be obtained as well as it does not result in pain in obtaining sample (urine). It is rapid with results ready within 15- 25minutes in all study subjects than in microscopy which ranges between 45 – 60 minutes and most times are not readily available to the doctors until after 24 hours. These duration are largely due to high outpatient case load and erratic power supply.

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