

Evaluation of Urine Malaria Test [umt] Kit and Blood Microscopy for the Diagnosis of Malaria in Febrile Individuals

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ABSTRACT

This study investigated two different diagnostic methods for malaria detection which are blood microscopy and Urine Malaria Test Kits (UMT). Malaria has been diagnosed in time past using clinical diagnosis which is based on the patient symptoms and physical findings at examination, the symptoms of malaria are often found in other diseases, likewise, the physical findings are often not specific. Recently, blood microscopy has become the gold standard method for malaria diagnosis. In order to minimize the invasive nature of the blood microscopy techniques, especially for on field detection, adapting non-invasive methods of biological sample collection for rapid diagnosis of malaria infections may provide a more efficient approach to case management and epidemiological studies of malaria. This study evaluates the detection of Plasmodium falciparum Histidine-rich Protein II (PfHRP-2) in urine samples and optimization as diagnostic markers for P. falciparum infection. Blood samples of febrile patients were used and tested for the presence of P falciparum through microscopy. In addition, urine samples of these patients were analyzed for PfHRP-2 using the Fyodor Urine Malaria Test kits. The diagnostic performances of the UMT kit and blood microscopy techniques were evaluated. Overall, the UMT results were thirty two (32) 64% positive, ten (10) 20% trace, seven (7) 14% negative and one (1) 2% of the patient was unable to void urine. While for the blood microscopy, forty three (43) 86% patients were positive while seven (7) 14% patients were negative. Findings revealed that UMTs have promising capacities for malaria diagnosis and require more optimizations to meet required diagnostic standards.

Keywords: *Epidemiological studies, febrile, histidine-rich protein II, Plasmodium falciparum, urine malaria test kit.*

INTRODUCTION

Malaria infection is a major worldwide health concern and the world's leading cause of death (WHO, 2018). Almost half of the world's population, or 3.2 billion people, are at risk of developing malaria (Onwujekwe *et al.*, 2009) (Mwanbingu *et al.*, 2019).

Guidelines for the detection, identification, and quantification of malaria parasites in research settings using thick and thin blood smears have been developed by the World Health Organization (WHO) (Njunda *et al.*, 2013), In order to diagnose malaria,

microscopy is primarily utilized as the gold standard. It also functions as a reference method for other malaria diagnostic procedures. (Slater *et al.*, 2011)

The objective of providing diagnosis for malaria to all people has recently been achieved due to several new researches on using body fluids that are under development for the diagnosis of malaria. These Novel technologies target parasite antigens (e.g. fluorescents rapid diagnostic test (RDTs) and hemozoin, a point of care for spectroscopy, and serology, which is currently commercially available as ELISA tests in blood bank screening in developed countries (Moody, 2023).

Microscopy has been the usual clinical practice for the diagnosis of malaria in endemic regions but this method of diagnosis is faced with so many issues like expertise, the quality of field microscopy, epileptic electricity supply and poor reagents among others (Rypien *et al.*, 2017), all efforts in reducing global malaria have led to no significant progress between (2015-2017). Although, detection thresholds of 4–20 parasites per microliter are achievable using a Giemsa-stained thick blood film in controlled laboratory situations, thresholds of 100–200 parasites per microliter are more common in field settings. (Wellington *et al.*, 2017)

Apart from RDTs, the urine malaria test (UMT) is a recombinant monoclonal antibody-based test that detects *P. falciparum*-specific HRP-2, a poly-histidine protein or fragment present in the urine of febrile patients (Adesanmi *et al.*, 2011) a specific urine-based malaria strip, the Fyodor UMT, is the only available UMT strip test in the Nigerian market. Considering the ease of use of RDTs and UMTs and according to the WHO recommendation of T3 (testing, treatment, and tracking of malaria), it is considered important to incorporate both tests in malaria diagnosis. Therefore, the adoption of community-based diagnosis using RDTs and UMTs, in addition to traditional hospital microscopy, has become imperative in malaria management. In this study, the evaluation of the effectiveness of two malaria diagnostic method using microscopy and UMTs was determined, also the comparative analysis between these two diagnostic methods were ascertained. (Kifude *et al.*, 2020)

The findings in this study may serve as a useful basis for health policies in malaria management. Rather than in a hospital, our findings can also help promote the implementation of UMTs at point-of-care facilities for self-malaria diagnosis and diagnosis at the community level.

The identification and characterization of proteins produced during human infection with *plasmodium spp.* have guided the malaria community in research diagnosis, epidemiology, and other efforts recently developed methods for the detection of these proteins [antigens]in the laboratory has provided new types of data that can inform the evaluation of malaria diagnostics, epidemiological investigations, and overall malaria control strategies. Here, the focus is primarily on antigens that are currently known to be detectable in human specimens and on their impact on the diagnosis of malaria in human populations.

MATERIALS AND METHODS

Sample Site:

A part of the study was carried out in Police Hospital Polac Kano, which is located in the tropical region of northern Nigeria. The region is endemic for malaria and has a year-round transmission of the disease. A continuous study was conducted in a Police Hospital in Ado Ekiti between the periods of September 2023 to January 2024.

Sample Collection Criteria

The inclusion criterion was as follows: patients of all ages visiting the emergency ward, and the outpatient department with acute febrile illness presenting with documented temperatures of $\geq 37.5^{\circ}\text{C}$ or a history of fever persisting for 2–4 days. Demographic information such as age, gender, symptoms of disease as well as physical examinations were also obtained from the participants.

The exclusion criterion was as follows: patients of all ages visiting the pediatric ward, medicine ward, and the emergency ward other than acute febrile illness, patients of all ages who have not treated malaria in the last 2-14 days. Patients with a history of hematuria, $>15/\mu\text{l}$ leucocytes and urobilinogen of ≥ 1 mg/dl in their urine since these parameters are features of many kidney diseases with probable high levels of antibody that may cause false positive results, those who were presenting with signs and symptoms of Rheumatoid Arthritis (painful joints, inflamed joints, limitation in motion, malaise, and tenderness of the joints) since Rheumatoid Arthritis has been proven to give False Positive results for RDT and patients who were already on antimalarial drug. (Biswas *et al.*, 2021)

Subsequently, the matched capillary blood drawn from heel/finger were stained and tested according to the WHO standard microscopy technique (WHO, 2022) and urine samples voided into universal bottles were tested with UMT Kits and the procedure on the leaflet duly followed in order to detect malaria parasites and to differentiate (when possible) the various species, while specially checking for *P. falciparum*.

Sample Size:

The study population comprised of 50 patients that met up with the inclusion criteria within 5 months. Daily average of 2 patients was used for the project work because they were highly febrile.

Sampling Technique:

The systematic random sampling technique was used, with daily attendance as sampling frame. Recruitment of participants was done daily from Mondays to Fridays in the Polac Clinic Kano and Police Hospital Ekiti. Furthermore, each participant was given an identification number so as to avoid confusion in the course of the study.

Sample Collection:

Blood samples from patients presenting with documented temperatures of $\geq 37.5^{\circ}\text{C}$ (99.5°F) or history of fever persisting for 2–4 days were drawn via heel during the period of peak fever, i.e., $38.9\text{--}40^{\circ}\text{C}$ ($102\text{--}104^{\circ}\text{F}$). Sample collection lasted for a period of 5 months, i.e., September 2023 to January 2024 (Osei *et al.*, 2021). Capillary blood was collected by finger pricking. 50 μL of this blood was used to make a thin and thick

blood film. Microscopic analysis was prioritized over other methods of malaria parasite determination. Urine was collected in a leak-proofed container for UMT analysis. (Achary *et al.*, 2015)

Malaria Blood Microscopy Procedure:

A blood specimen collected from the patient was spread as a thick or thin blood smear, stained with Giemsa and examined with a 100X oil immersion objective (Kosack *et al* (2013). Visual criteria were used to detect malaria parasites and to differentiate (when possible) the various species, while specially checking for *Plasmodium falciparum*. (WHO, 2023)

Urine Malaria Tests Procedure:

The urine test was performed by dipping the UMT strip in 200 µL of urine for two minutes to allow the sample to wick and saturate the strip. The strip was then retrieved, placed on its foil pouch packaging and incubated for 20 minutes Amodu *et al.*, (2022). The results were reported as negative, positive, or trace, if is not too visible: 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 even.

RESULTS AND DISCUSSIONS

Results:

The UMT results were 64% positive, 20% trace, 14% negative and 2% patient was unable to void urine. While for the blood microscopy, 86% patients were positive, while 14% patients were negative.

Table 1: Distribution of patient’s population by Gender

PARAMETERS	NUMBERS
MALE	28
FEMALE	22
TOTAL	50

Table 2: Distribution of study population according to age

Age Range	Population Distribution
<20	8
20-29	13
30-39	25
40-49	3
>50	1
Total	50

Microscopy Malaria Test Results

Forty three (43) 86% of 50 samples were positive with microscopy while seven (7) 14% of the samples were negative.

Table3: Showing Blood Microscopy Result

PARAMETERS	BLOOD MICROSCOPY RESULT
POSITIVE	43
NEGATIVE	7
TOTAL	50

Urine Malaria Test Result

Forty two (42) results were found positive by the UMT. Therefore, total positive is 84% for UMT while (8) 16% were negative.

Table 4: showing the results obtained using the UMT kit

Parameters	UMT Results
Positive	32
Negative	7
Unable to void urine	1
Trace	10

DISCUSSION

From this study, there was a close agreement between the UMT when compared to blood microscopy with 84% and 86% respectively. The UMT has shown a high level of efficiency in testing urine samples of malaria patients obtained, other studies as well with non-blood samples showed comparatively promising results, however they require further improvement (Harchut *et al.*, 2013). There could be many reasons for some inconsistencies in the UMT results, which may be related to parasite antigen production, antigen content in urine and time of void (Genton *et al.* 1998). Genton *et al.* (1998) noted that the amount of malaria antigen was low in urine and dependent probably on the time of collection of the samples they suggested that first void morning urine might probably give better sensitivity than later timed samples. This may not be practicable in clinical practice where the results are required for immediate treatment. With the probable variability in malaria antigen quantity, it is likely that the expected amount of antibody impregnated in the urine-specific test kits as well as the quantity of body fluid required may be higher than those of blood-specific test kits thus necessitating a probable further optimization of the Fyodor UMT to enhance test results in low parasitemia. It is known that the characteristics of the antibody impregnated in the nitrocellulose pad of the immunochromatographic test kits also determine the sensitivity. For example, the monoclonal or polyclonal antibodies affect the malaria UMTs at higher parasite densities (≥ 200 parasites/ μl of blood).

The degree of false positive results especially among children may suggest that the UMT is able to maintain acceptable level of false positive results particularly in areas of high malaria transmission (Choon *et al.*, 2017). Also, the false positivity related to the presence of the gametocyte is indicative of the ability to detect sexual form of *P. falciparum* a factor which is useful in absolute tests against the clinical episodes that was used in this study. However, in areas of low malaria endemicity, this level of false positives may create drug wastage, which the current malaria control efforts seek to reduce. False positive results may be attributed to the ability of all histidine-rich protein 2 (HRP2) antigen malaria test kits to detect the parasite antigen even after malaria illness. The presence of rheumatoid factor and schistosomiasis in a patient may also lead to false positivity, and will need to be further evaluated. These factors are known to affect the blood type malaria UMTs, but little is known about such influence on the

urine malaria test kits. It may be assumed that since both (blood and urine-based) test kits are specific for HRP2, such effect may also occur with the UMT. (Galan *et al.*, 2023) Some of the limitations observed with the use of the urine malaria test kit were the high colour degradation of the test line within an hour after testing. This is a disadvantage, which makes it difficult to crosscheck the results in cases of doubt. The delay in provision of some subjects, particularly among children, may be a delay factor in the promptness of testing and treatment. The sample size may also be a factor in the resultant accuracy result, which may probably be answered by the completed larger clinical trials.

CONCLUSION

From our research findings we can finally conclude that the results from both blood microscopy and UMT showed very close similarities, although there are prospects that the UMT kit can undergo further development and evaluation, which can lead to further improvement particularly in the area of test stability. In future, the kit can attain advanced optimization in the detected areas of deficiency which will enhance the usefulness of the non-blood-based kits as a reliable and easy to use alternative tool in the diagnosis of malaria in the areas where the competence for blood microscopy is not reliable, and safety concerns are expressed.

Some of the limitations observed with the use of the UMT were the delay in provision of urine by some subjects, especially among children. This may be a delay factor in the promptness of testing and treatment. The prevalence of malaria for our study was limited to a single plasmodia species; *Plasmodium falciparum*, hence not revealing the true prevalence of malaria in the community as there could be infections with other plasmodium species. The Urine Malaria Test kit that was evaluated showed a lot of similarities with blood smear microscopy as gold standard. Hence, it can be used in our setting for the prompt and accurate diagnosis of malaria in febrile patients.

CONFLICT OF INTEREST: The authors declares no conflict of interest

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