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# PREVALENCE OF MALARIA USING TWO RAPID DIAGNOSTIC TEST KIT IN THE DIAGNOSIS OF MALARIA IN GOMBE STATE, NIGERIA.

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## Abstract:-

*This study aims at evaluating the comparative diagnostic efficiencies of two commercially available kits in relation to microscopy in detecting Plasmodium falciparum HRP-2, a polyhistidine antigen in urine and blood of febrile patients for malaria diagnosis along with blood based RDTs. This was an observational study in which matched blood and urine from symptomatic patients were tested for malaria using two rapid tests, with microscopy as gold standard in the outpatient clinic of Federal Teaching Hospital Gombe, Gombe state North Eastern Nigeria were enrolled who presented with fever (≥37.5°C). Thick films were prepared, stained and examined for malaria parasite under the microscope using the oil immersion objective while urine samples as well as blood were taken and immediately tested using RDTs.*
*With the blood smear microscopy as standard, the disease prevalence was 68.4% and sensitivity for the UMT and SD Bio line (Blood RDT) was 70.1% respectively. The RDT had a specificity of 96.3% as well as the positive and negative predictive values were 97.6% and 59.7% respectively. The rapid diagnostic kits showed moderate level of sensitivity compared with blood smear microscopy as they show considerable acceptable levels in the diagnosis of malaria.*

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

## INTRODUCTION

Malaria is considered one of the most important of all tropical diseases in terms of morbidity and mortality on a global scale. 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 [1]. Malaria infection is caused by four species which are *Plasmodium falciparum, Plasmodium malariae, P.vivax and P.Ovale.* An estimated 85% of cases and 90% of carriers (mainly asymptomatic) are found in tropical Africa. Twenty to Thirty (20 – 30%) deaths in children been attributed to the disease [2].

In Nigeria malaria due to *P. falciparum* is a major cause of morbidity and mortality, it accounts for 42% of all outpatient clinic visits, 8% of all certified deaths and 20% of under-five mortality [3, 4]. Malaria is the commonest cause of death in children aged 0-4 years [5]. The Global Malaria Control Strategy [6] acknowledges that prompt, rapid and accurate diagnosis is required to develop effective management strategies for prevention of severe and complicated disease. Although clinical diagnosis of malaria is relatively inexpensive to perform, fast, and requires no special equipment or supplies it may not be accurate enough [7]. This is because, it relies on identification of clinical signs and symptoms which may also be caused by several other diseases, including, typhoid fever, influenza, meningitis, hepatitis, as well as hemorrhagic fevers [8, 9].

Clinical diagnosis of malaria is based on a high level of presumption [10], following which the laboratory microscopic and/or other detective approaches are needed for confirmatory diagnosis. The conventional light microscopy is presently the established and most acceptable method for the laboratory confirmation of malaria [11, 12]. There are already available rapid test kits {immunochromatographic tests (ICTs)} for alternative diagnosis of malaria, these techniques involve the detection of malaria parasite antigens in body fluids (Blood, Urine). These mostly employ the use of a dipstick or test strip, bearing monoclonal antibodies against malaria parasite antigens in immune-chromatography. Immuno-chromatographic tests do not need any specialized expertise, and can be performed within 15 to 30 minutes. Theses antigen based dipstick is field applicable and does not require any technical skill to perform.

Rodriguez-del Valle *et al*. (1991), detected *Plasmodium* antigens and anti-malarial antibodies, in the urine of infected individuals and suggested that a urine-based assay for diagnosis of malaria would be feasible. It is in a bid to improve upon the limitations of the current diagnostic tools this study proposed to study the comparative effectiveness of rapid urine-based fieldapplicable dipstick and blood based test kits for the diagnosis of malaria in infected individuals using *Plasmodium* antigens excreted in the urine and blood of infected subjects.

Existing immunochromtographic tests for malaria also have the weaknesses of invasive tests including exposure of health-workers to blood [13] and poor acceptance by some communities. Indeed, currently, none of the major tests for diagnosis of malaria can function without blood as a test material. This characteristic makes all these major tests unsuitable for homebased management of malaria (HBMM) in most of the low-income rural communities where most malaria-related deaths and morbidities occur. Perhaps, this is partly because, blood is used for pacification of gods and other rituals in these communities and therefore considered fetishism to engage in any practices that have connections with blood [14]. With the connection of HIV/AIDS to blood, most people, would, under normal circumstances, do not want to have anything to do with blood. In communities with inadequate healthcare infrastructure, simple field-applicable rapid diagnostic tools for malaria in pregnancy would lead to early detection and treatment of pregnancy malaria at the community and home-based level timely enough to avoid complications and/or death that could result. Therefore, a diagnostic technique, which combines high specificity, sensitivity, and rapidity to perform with non-invasiveness and cost effectiveness, would be a most welcome alternative.

To reduce these deficiencies, malaria Rapid Diagnostic Test (malaria RDT) kits are been developed for the rapid diagnosis of clinical malaria within the last 10 years. These RDTs help in early diagnosis and treatment thus reducing the morbidity and mortality, also contributes to a fast and right treatment. Rapid Diagnostic kits unlike microscopic diagnosis are less strenuous and faster as they do not require laboratory equipment and they have been developed in different test formats like dipsticks, strip card, pad wells or cassette. These kits use the antigen or enzymes derived from parasite that have infected humans by detecting for novel Plasmodium proteins which are secreted/ shed in urine or blood of febrile malaria patients (Histidine rich protein, HRP2) produced by *Plasmodium* during its asexual forms and early gametocytic stages. Preferred targeted antigens are those which are abundant in all asexual and sexual stage of the parasite.

The use of rapid diagnostic test (RDTs) for malaria is becoming relevant in malaria diagnosis. In endemic areas like Nigeria, with varied shortcoming such as unstable power supply to power the microscope, none dedicated microscope for malaria testing and over worked microscopists has greatly increased the TAT (Turnaround time) of malaria testing in which results are available after 24hrs to the clinician thus prompting for prescription of antimalarial drugs irrespective of the outcome of the laboratory testing for malaria, RDTs however take a minimum of 25min - 30min to detect if fever is caused by malaria parasite by detecting the antigen within the subjects body fluids obtained (urine or blood).

## MATERIALS AND METHODS

The research was carried out in Gombe, Gombe State in the outpatient ward of the Federal Teaching Hospital (FTH) the primary health care facility in Gombe town. With a sample size of 171 subject aged 1 to > 21 years were recruited after

satisfying the criteria for inclusion for the study after presented themselves with cases of fever and other related symptoms like headache, body weakness, chills, joint pains, cough, convulsion etc.

**SAMPLE COLLECTION**

For each subject after successful enrollment for the study are either finger pricked or blood drawn with syringes and placed in EDTA bottles in the laboratory for microscopic analysis as well as for blood RDT test, finger pricking was done with two drops of blood(5µl) placed in the sample well and 4 drops of assay diluent placed in the assay diluent well, while for UMT, urine samples will be collected from same patients in universal bottles, marked and immediately tested in accordance with W.H.O standards [15].

**Urine Sample testing principle.**

Testing is carried out when a test strip with UMT Kit (Lot No.7014978, Fyodor Biotechnologies, and 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 according to manufacturer's instruction on the kit insert.

**SD Bioline malaria antigen *P.f* rapid test procedure.**

Testing is carried out when a test strip with Kit (Batch No.05CDA022A, Standard diagnostic INC) The patients' finger was cleaned with alcohol swab and allowed to dry before pricking using a lancet to get blood. About 5 µl of blood was dropped into the round sample well of the test strip and 4 drops of assay diluent were added into the square assay diluent well and the results read after 15minutes according to manufacturer's instruction on the kit insert.

**Smear Preparation**

The capillary blood samples drawn from study subjects will be used to prepare thin and thick blood smear for microscopy to diagnose for malaria parasite [15]. Using a tunicate tied to the upper cubic-forsa of the left arm, a 5ml syringe was used to draw 2ml of venous blood and placed in EDTA bottles. 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 due to a number of viral, bacterial and parasitological diseases that can be transmitted through the blood [15].

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. The thin film was then fixed in absolute methanol for 2 sec to prevent lysis of the red blood cell and allowed to air dry on the rack while the thick film was not fixed. The slides are then stained with 3% Giemsa prepared in pH 7.2 and allowed to stand dry for 45 min and rinsed in clean water and then allowed to air dry on the slide racks to drain dry for laboratory reading of slides in accordance with W.H.O standard microscopy technique [15 and 16]

**Microscopic Examination**

Microscopic examination of the blood films were done using the X100 oil immersion objectives. Two trained reference microscopists who were blinded to each other's results and the allocation of the patient double read the slides using light microscopy using a minimum of 100 fields on the thick film to ascertain the slide is negative for malaria parasite. They defined a slide 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.

**RESULTS**

Table 1 shows the overall distribution of patients in relation to age and sex with 9 (5.3%) infected within ages 0-5, 22 (12.8%) infected under ages 6-10, 33 (19.3%) under ages 11-20 and 53 (30.9%) were infected under ages above 21 years old. In general 95 males and 76 females were tested respectively. These represents 55.6% and 44.4% respectively. The rate of detection of malaria diagnosis on the various RDTs shows slight difference as compared to microscopy.

**Table 1: Overall Distribution of patient by age and sex attending the outpatient ward of Federal Teaching hospital Gombe screened for malaria with HRP-2 test kits.**

| Age (Class interval) | Gender | | Frequency (%) | Positive UMT (%) | Positive Blood RDT (%) | Positive Microscopy (%) |
|---|---|---|---|---|---|---|
| | Male | Female | | | | |
| 1-5 | 9 | 8 | 17 (10) | 8 (4.7) | 9 (5.3) | 9 (5.3) |
| 6-10 | 16 | 10 | 26 (15.2) | 14 (8.2) | 14 (8.2) | 22 (12.8) |
| 11-20 | 20 | 28 | 48 (28.1) | 25 (14.6) | 25 (14.6) | 33 (19.3) |
| >21 | 50 | 30 | 80 (46.7) | 37 (21.6) | 36 (21.1) | 53 (30.9) |
| **Total** | **95** | **76** | **171 (100)** | **84 (49.1)** | **84 (49.1)** | **117 (68.4)** |

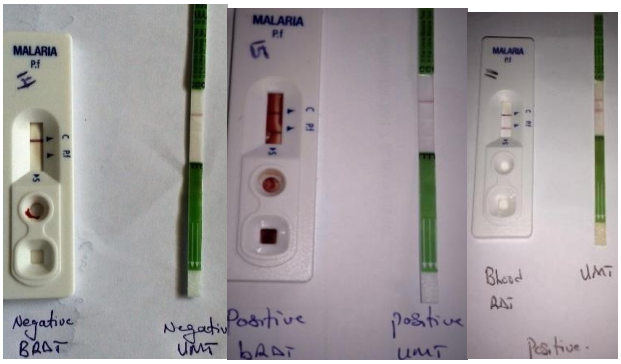


**Figure 1: Negative and positive RDT for Plasmodium falciparum using two different test matrix RDTs.**

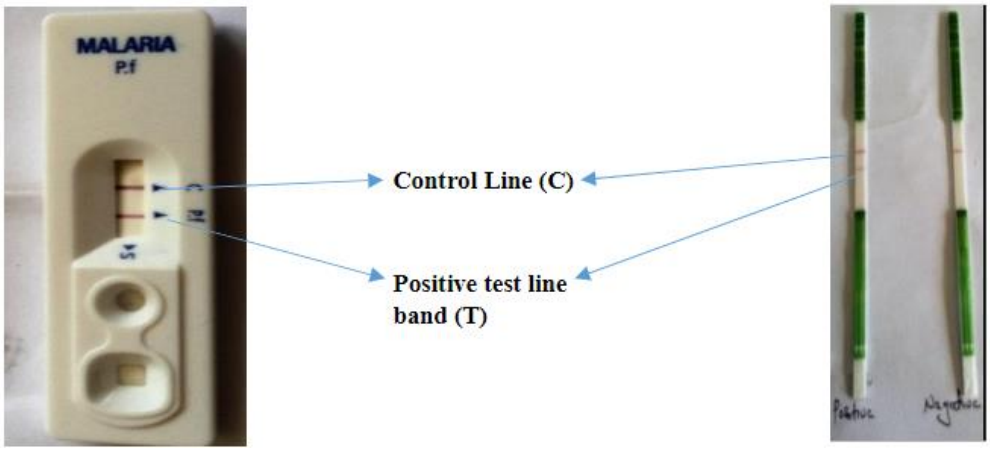


**Figure 2: Schematic representation of both SD Bio-line Blood RDT and UMT test showing both positive and negative result.**

**Table 2: Comparison of the two RDTS with microscopy.**

| RDTs | Microscopy | | |
|---|---|---|---|
| **SD Bioline *Pf*** | **Positive (%)** | **Negative (%)** | **Total (%)** |
| **Positive** | 82 (47.9) | 2 (1.2) | 84 (49) |
| **Negative** | 35 (20.5) | 52 (30.4) | 87 (51) |
| **Total** | **117 (68.4)** | **54 (31.6)** | **171 (100)** |
| **UMT Test Kit *Pf*** | | | |
| **Positive** | 82 (47.9) | 2 (1.2) | 84 (49) |
| **Negative** | 35 (20.5) | 52 (30.4) | 87 (51) |
| **Total** | **117 (68.4)** | **54 (31.6)** | **171 (100)** |

$p<0.05$

Table 2 shows the comparative result of the two diagnostic test kits showing that both test kit using different test samples (blood and urine) gave a true positive value (82, 47.9%) respectively, false negative values (35, 20.5%) while the false positive for both UMT and SD Bioline (2, 1.2%). The true negative results were 52, 30.4% for UMT and SD Bioline respectively.

Table 3 gives the variation in sensitivity of the two rapid diagnostic test kits by parasite densities which gives a detectable level of 88.2% respectively for Urine malaria and SD Bioline RDTs. While at varying parasite densities gives a 78.9% - 100%.

**Table 3: Variation of sensitivity of the two rapid diagnostic test kits by parasite density.**

| Parasitaemia (parasite/µl) | No. Positive by microscopy (%) | No. Positive by UMT RDT. (%) | No Positive by SD-Bioline RDT. (%) | Sensitivity (%) | |
|---|---|---|---|---|---|
| | | | | UMT | SD Bioline |
| *≤100(+)* | 17 (14.5) | 0 | 0 | 0 | 0 |
| *≤200 (++)* | 34 (29.1) | 30 (35.7) | 30 (35.7) | 88.2 | 88.2 |
| *≥201 (+++)* | 57 (48.7) | 45 (53.6) | 45 (53.6) | 78.9 | 78.9 |
| *≥500 (++++)* | 9 (7.7) | 9 (10.7) | 9 (10.7) | 100 | 100 |

**Table 4: Diagnostic evaluationof Malaria RDT**

| Performance characteristics | RDT method (UMT) | SD Bioline |
|---|---|---|
| **Sensitivity** | 70.1% | 70.1% |
| **Specificity** | 96.3% | 96.3% |
| **Positive Predictive Value** | 97.6% | 97.6% |
| **Negative Predictive Value** | 59.7% | 59.7% |
| **False Negative Rate** | 29.9% | 29.9% |
| **False Positive Rate** | 3.7% | 3.7% |
| **Efficiency (Accuracy)** | 78.4% | 78.4% |

Table 4 shows the general performance characteristic of the rapid diagnostic test kits using different test matric in the diagnosis of malaria Plasmodium falciparum HPR-2. Table 5, gives the distribution of malaria parasite in relation to age and gender.

**Table 5: Distribution of malaria parasite in relation to Age and Sex of patients attending the Federal teaching hospital Gombe**.

| Age | No Examined (O) | | Number Infected (E) | | O-E | | $(O-E)^2$ | |
|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | Male | Female |
| **1-5** | 9 | 8 | 5 | 4 | 4 | 3 | 16 | 9 |
| **6-10** | 16 | 10 | 12 | 10 | 6 | 0 | 36 | 0 |
| **11-20** | 20 | 28 | 11 | 12 | 8 | 16 | 64 | 256 |
| **>21** | 50 | 30 | 35 | 18 | 15 | 12 | 225 | 144 |
| **Total** | **95** | **76** | **63** | **44** | **33** | **31** | **341** | **409** |

$\chi^2$ Cal = 9.29 female, 5.41 male.
p- value $\chi^2_{Tab}$ = 7.815 ($p<0.05$) showing that no significant difference in infection among sex and ages since $\chi^2$ cal value in male is lower than $\chi^2$ tab value at $P<0.05$ and female $\chi^2$ cal value is greater than the $\chi^2$ tab value and degree of freedom 3 respectively.
*Prevalence rate is expressed as % age of no. positive divided by no examined. = 68.4%

**Discussion**

From the study, the comparative study of the two rapid diagnostic test kit as compared with blood microscopy shows that it had an overall sensitivity of 70.1% respectively and specificity of 96.3% respectively in the diagnosis of malaria parasite among febrile patients. The study shows the ability of the test kit strips in detecting the antigen of *Plasmodium falciparum* (HPR-2) in the urine of febrile patients as well as in the blood at reliable levels of 78.4% (kappa= 0.5673). The prevalence of parasitic infections among the different age groups in the present study was not significant ($P< 0.05$) indicating that the occurrences of these infections on these age groups were the same. The present study has shown that *Plasmodium* infections were more common in the male than in the female subjects (Table 5).

This study reveals that the prevalence of malaria caused by *P. falciparum* in Gombe was 68.4%, the use of RDTs are generally important as a complimentary diagnostic tool of malaria in malaria case management in the country and no significant association between sex and age in rate of infection by malaria parasite. It is therefore necessary to advocate

for greater use of RDTs as point of care testing which should be complimented with laboratory test for diagnosis of malaria in our community as this practice will enhance adequate and prompt diagnosis of acute febrile illnesses. A well-coordinated enlightment and educational programs aimed at malaria control and prevention should be instituted by the authorities concern in Gombe State as well as in the North Eastern Nigeria.

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