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Research Article

A Comparison of Blood Smear Microscopy, Rapid Diagnostic Test (RDT), and Urine Malaria Test (UMT) for the Diagnosis of Malaria in Children Aged ≤ 5 Years

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Abstract

Background: Malaria is a major global public health challenge. Diagnosis is a critical link to mitigating its impact. New malaria diagnostic methods that simplify malaria testing, might improve acceptance, access to tests, and compliance with the test before treatment policy of WHO. While microscopy and RDT are established, UMT is novel and merits further evaluation.

Methods: This was a cross-sectional diagnostic test study, 262 children with presumptive malaria were recruited. All were tested with the three methods: microcopy, RDT, and UMT.

Results: The prevalence of malaria using microscopy, RDT, and UMT test methods were 33.59% (88), 20.99% (55), and 2.67% (7) respectively. Only fathers' educational status showed a significant association with malaria test outcomes (i.e., positive or negative). The sensitivity of RDT was 29.5%, and its specificity was 83.3%. It's positive predictive and negative predictive values were 47.3% and 70% respectively. UMT recorded a sensitivity of 4.5% and specificity of 98.3%, while its positive predictive and negative predictive values were 57.1% and 67.1% respectively.

Conclusion: The UMT, though preferred by the patient's guardians, had lower sensitivity and specificity compared to microscopy and RDT. More research is still needed to make UMT robust for clinical practice use.

Keywords: Malaria; Giemsa Microscopy; Rapid Diagnostic Test; Urine Malaria Test Kits

Abbreviations

UMT: Urine Malaria Test Kit; RDT: Rapid Diagnostic Test Kit; WHO: World health organization; NMIS: Nigeria Malaria Indicator Survey

Introduction

Malaria is a global health challenge. The importance of definite diagnosis as a component of optimal case management of malaria cannot be over-emphasized. The World Health Organization (WHO) recommends testing before treating as most ideal [1]. However, a presumptive diagnosis is still widely practiced, with the attendant risk of the emergence of drug resistance, unwarranted exposure to adverse drug reactions, and delay in seeking appropriate care in cases of wrong diagnosis [1]. The reliability and accuracy of microscopy for diagnosis are likely to be affected by the skills and logistics required to deliver these services, especially in resource-poor settings [2]. On the other hand, while the use of RDT requires less skill, it is also invasive like microscopy requiring blood sampling and safety measures in handling blood waste. Quick and efficient

diagnosis of malaria will facilitate case management and thereby reduce morbidity and mortality [3]. The UMT which is promoted as a non-addictive reagent, no blood, no equipment one-step approach to diagnosis can improve the speed and convenience of diagnosis [4]. The UMT is reported to be a fast, non-invasive point-of-care test with similar sensitivity to the WHO-approved RDTs [1]. Thus, UMT is a welcome addition to the available diagnostic options. However, since few studies have been published, with very promising results and variable recommendations for its use as a non-invasive but dependable method of diagnosis, it is our humble opinion that a direct evaluation of its utility compared to microscopy and RDT will provide additional information on its usefulness in children under 5yrs, who are suffering high morbidity and mortality generally [4,5].

Therefore, this is a comparative study that sought to evaluate the performance and caregiver preferences regarding the available options for malaria diagnosis.

Material and Methods

Study area

The study was conducted in Garki Hospital Abuja (GHA), Abuja, which is situated in North-Central Nigeria. Garki Hospital Abuja is the oldest public hospital in the FCT and was created in 1986. She got her name from the area she was located which is the Garki district of FCT. The hospital which is privately run presently provides both in-patient and outpatient services and serves as a tertiary referral center.

Study design

The study was conducted in the paediatrics outpatient clinic (POPC) of Garki Hospital. Inclusion criteria were age ≤ 5 years with a presumptive diagnosis of malaria and axillary temperature ≥ 37.5 C or fever in the last 24 hours.

Ethics considerations

Ethical approval was obtained from the Ethical Review and Research Committee of the Hospital Management Board, Federal Capital Territory Abuja. Informed consent was obtained from the parents or guardians of the children. Participation was voluntary and guardians could withdraw their children at any time from the study without prejudice to further treatment. Those with evidence of malaria were treated with artemisinin combination therapy according to the Nigerian national guidelines on malaria treatment [1].

Study procedures

Children on arrival at the POPC, had their vital signs taken by Nurses and subsequently referred to Doctors for consultations (Research assistants). Children ≤5 years diagnosed with presumptive malaria by the consulting Doctors were referred to the Investigator who introduced the study to them, obtained consent from willing guardians, and assessed patients for eligibility before enrolment. None consenting caregivers had their wards referred for routine care. The first enrollee was selected by random sampling from the pool of the first four. Subsequently, every fourth patient was recruited systematically. Patients who met the inclusion criteria were enrolled and blood and urine samples were tested for malaria.

Laboratory investigation

Urine samples were collected into a clean universal container. The urine samples were screened for leukocytes and nitrites to exclude urinary tract infection as an alternative or contributory cause of fever, and urobilinogen and haematuria which reduce the specificity of UMT. Two patients who had prostration suggestive of severe malaria, four who had nitrite in urine, and 20 who had received ACT within five weeks of presentation were excluded.

Enrolled subjects were interviewed using a structured questionnaire. The socio-demographic data, history of fever, headache, chills and or rigors, joint aches, weakness, anorexia, bitter taste, vomiting, convulsions, abdominal pains, ear pain, cough, diarrhea, and history of use of antimalarial for more than five weeks. Axillary temperature was recorded to the nearest 0.1 °C using a digital thermometer. The subjects were examined for pallor, jaundice, lethargy, respiratory rate, pulse rate, splenomegaly, enlargement of the liver, and any other abnormality.

The urine sample of enrolees was tested using the UMT. The Fyodor biotechnological UMT strip was dipped into the urine sample and allowed to stand for 25 minutes. Two lines on the test strip indicate a positive test. One line (if control), one line (if

test), and no line are interpreted as negative, invalid, and invalid, respectively. All invalid results were repeated and if still invalid enrolee was removed from the study.

Each subject's middle finger or heel was disinfected with methylated spirit-soaked cotton wool and a sterile lancet was used to prick. Blood was expressed from the finger/heel prick directly onto the one-half slide for thick film, and the other half of the slide for thin film smear [6].

The blood slides were stained using 10% Giemsa stain and were read with ×1000 magnification (with oil immersion) by two trained microscopists working independently. Positive slides were those with asexual parasite forms identified by both microscopists. Plasmodium parasitemia was estimated based on the assumption that 8,000 leucocytes have an estimated volume of 1 µl, which is the arbitrarily accepted figure [7]. The number of white blood cells (WBCs) was counted and parasite density was estimated as follows.

Parasite density/ µl = (Number of parasites × 8000)/Number of leucocytes [7].

If less than 100 parasites were counted in the first 100 WBCs, counting was continued to 200 WBCs; if less than 100 parasites were counted, the counting was further extended to 500 WBCs [6]. A blood film was reported negative if 100 thick smear film fields did not show asexual forms after counting to 500 WBCs [6]. The average of the parasite density measured independently by the two microscopists with a discrepancy of ≤ 20% was adopted, while a discrepancy > 20% was resolved by a third microscopist in which case the average of the slides with a parasite density of two slides with discrepancy of ≤ 20% was adopted. Thin film blood slides were microscopically inspected for species differentiations [6].

Blood for the RDT was collected using a micro-pipette for blood microscopy. A drop of fresh blood was deposited into the well of the RDT cassette, two drops of the clearing buffer were applied to the buffer well and the cassette was left to stand on a flat surface. According to the instruction manual, the Aria Malaria Pf/Pan rapid test was read at 15 minutes. The presence of at least the control line and any other line was interpreted as a positive result, while the control line only was considered a negative. When the control line was absent, but either or both of the other lines were positive, the result was considered invalid, and the test was repeated.

Statistical analysis

Data were entered, cleaned, and analysed in Epi Info™ version 7.2.0.1 (CDC, Atlanta, Georgia, USA). Additional analyses were done using OpenEpi statistical calculator. Descriptive statistics are reported as frequencies, means, (or medians), and standard deviations of the subject characteristics. The microscopy, RDT, and UMT results are reported as proportions. The sensitivity, specificity, and positive and negative predictive values of the RDT and UMT were calculated using blood smear microscopy test results as the reference test. The degree of agreement between RDT and UMT and between the UMT and blood microscopy and the RDT and a blood smear was calculated using Cohen's kappa. In addition, a logistic regression model was used to explore the association between a positive malaria test and patient characteristics while controlling for the effect of confounding variables. A p-value of ≤ 0.05 was considered significant in all analyses.

Results and Discussion

Results

A total of 262 febrile children < 5yrs old were recruited. There were slightly more males 133 (50.8%) than females 129 (49.2%) with a mean age of 32.6 ± 14.6 SD months, the majority (30.7%) being in the age group 36-47 months. The least numbered group aged ≤12 months was 11 (4.2%).

Malaria prevalence was 33.59% (88), 20.99% (55), and 2.67% (7) using blood smear microscopy, RDT, and UMT respectively. See Figure 1. The only species detected was *Plasmodium falciparum.*

Figure 1: Malaria Prevalence using different diagnostic methods.

The mean parasite density was 8,063.4 ± 60.9 parasites/ µL with 18, 36, 11, 7, and 16 study subjects having parasite counts/ ul of 1-49/ul, 50-199ul, 200-499/ul, 500-4999/ul and ≥5000/ul respectively.

At parasite density < 50 parasites/µl, out of 18 (100%) persons who had detectable parasitemia on thick film microscopy, RDT was only able to detect 1 (5.6%) of study subjects. At a parasite density of 50 - 199 parasites/µl, out of 36 (100%) study subjects who had this level of parasitemia on thick film microscopy, RDT was only able to detect 1(2.8%). At parasite density 200-500 parasites µl, out of 8 (100%) study subjects who had this level of parasitemia on thick film microscopy, RDT was only able to detect 3(37.5%) At parasite density 500 -4999 parasites/ul, out of 7(100%) study subjects who had this level of parasitemia with thick film microscopy, RDT was only able to detect 5 (71.4%) At parasite density > 5000 parasites/ul, RDT was able to detect all 16(100%) study subjects who had parasitemia with thick film microscopy. This demonstrated that detection increased as parasite count/ul increased.

At parasite density < 5000 parasites/µl, UMT was unable to detect all persons who had parasitemia with thick film microscopy. UMT was only able to detect malaria in patients with a parasite count > 5000 parasites/µl.

The sensitivity of RDT was 29.5% (20.3-40.2), and its specificity was 83.3% (76.9-88.5). Its positive and negative predictive values were 47.3% and 70% respectively.

UMT recorded a sensitivity of 4.5% and specificity of 98.3%, while its positive predictive and negative predictive values were 57.1% and 67.1% respectively.

Test combinations	Sensitivity	Specificity	Positive predictive Value	Negative predictive Value
Microscopy-RDT	29.5%	83.3%	47.3%	70.0%
95% CI	20.3-40.2	76.9-88.5	36.1-58.8	66.8-73.1
Microscopy- UMT	4.5%	98.3%	57.1%	67.1%
95% CI	1.3-11.2	95.0-99.6	23.4-85.4	65.9-68.2

Table 1: The sensitivity, specificity, and predictive values of RDT and UMT.

	Rapid diagnostic test	
Variables	Positive n = 26 n(%)	Negative n = 62 n(%)
Levels of parasite density (parasites/ul)		
1 - 49	1(5.6)	17(94.4)
50 - 199	1(2.8)	35(97.2)
200 - 499	3(27.3)	8(72.7)
500 - 4999	5(71.4)	2(28.6)
≥ 5000	16(100.0)	0(0.0)

Table 2: Level of parasite density compared to RDT test outcomes.

	Urine Malaria Test	
Variables	**Positive n = 4**	**Negative n = 84**
Levels of parasite density(parasites/ul)		
1 - 49	0	18
50 - 199	0	36
200 - 499	0	11
500 - 4999	0	7
≥ 5000	4	12

Table 3: The association between parasite density level and UMT positivity.

Discussion

This study was designed to compare the performance of microscopy, RDT, and UMT (a newly introduced national guideline on malaria diagnosis, 2020) [1].

Studies comparing the performance of microscopy and RDT using blood in the diagnosis of malaria are numerous. However, only a handful of studies have done the same for UMT. This study revealed that at baseline there were significant diagnostic performance differences between the use of blood film microscopy, RDT, and UMT in screening for malaria infection.

Out of 262 subjects diagnosed clinically with malaria, malaria prevalence was 33.59%, 20.99%, and 2.67% with thick film microscopy, RDT, and UMT respectively. The only malaria specie identified was Plasmodium falciparum.

Dependence on a presumptive diagnosis result in over-prescription, delay in appropriate diagnosis, exposure to unnecessary treatment, and increases the risk of the emergence of resistant strains [1]. This discordance reinforces the test-before-treatment policy of WHO [8].

In the (NMIS2015), the prevalence of malaria in children under 60 months of age, residing in Abuja, Nigeria was reported to be 20.2% and 38.5% for microscopy and RDT respectively [9]. Malaria prevalence using microscopy as a standard was higher for this study compared to the NMIS 2015 findings, this may be due to the timing of the study, which was conducted between August and October, during the rainy season (peak transmissions), while the NMIS 2015 was conducted in October and November when the rainy season was effectively wounding up [9]. In addition, the NMIS 2015 reported malaria prevalence from RDT as higher than microscopy, which was reversed in this study [9]. This could have been accounted for by the fact that, in the NMIS2015, recruitment was not limited by exposure to ACT, thus no study subject was excluded on account of recent exposure to ACT unlike in the index study. The reported persistence of the HRP-2 antigen for ≤ 5 weeks in patients after the use of ACT is the possible explanation for RDT malaria prevalence being greater than microscopy in NMIS2015 [9]. While parasitemia, which microscopy detects can be cleared by ACT, HRP2 can persist in the blood of patients even after parasite clearance for weeks as earlier stated.

Notable is that only 18.1% of study subjects in this study had a parasitemia level of ≥5000, compared to 68% of subjects who had this level of parasitemia in the Lagos multicenter study, which may account for the better performance of UMT in the later study [5].

Variations in how UMT was used in various studies may have accounted for some variation in performance [2,4,5].

A false positivity of 16.7% for RDT was recorded in this study, above the value of the 10% false positivity rate recommended for RDT by WHO [8]. Thus reinforcing the need for, RDT results to be interpreted with caution following prior ACT exposure. A false positive value of 1.7% for UMT, may be due to low sensitivity and high threshold (>5000 parasites /μl) for UMT antigen detection found by this study.

Samal., *et al*. reported that RDT and UMT failed to detect malaria in 80% and 52 (96.3%) when parasite density was ≤200 parasites/μl respectively [10]. This suggests parasite density is an important factor in RDT and UMT sensitivity as shown in this study [10].

The ages were segregated into, 5 sub-groups; <12months, 12-23months, 24-35months, 36-47 months, and 48-<60months. The group 36-47 months had the most recruits and peak group prevalence in the study, with a plateau in prevalence in the 48-59 months group. This pattern was also reflected in a Papua New Guinea study and NMIS 2015 [8,10]. This phenomenon is explained by waning maternally acquire humoral immunity in <5yrs of life

and persistent parasitemia-induced active immunity subsequently from ages >3yrs [10-12]. This could also explain the plateau/slight drop in malaria prevalence noted from the study from ages 3-5 years.

Mykola Pinkevych., *et al*. demonstrated that, as maternal antibody-based immunity wanes, the child gradually acquires its immunity post-malaria exposure [13]. This phenomenon might explain the gradual rise and final plateau as in this study.

This study found no relationship between gender and malaria infection, as collaborated in NMIS2015, and Asaga., *et al*. in North Central Nigeria [3,8]. Nwaneli., *et al*. in Enugu reported that lower socio-economic class, lower maternal educational attainment, and residents of rural areas had a higher malaria prevalence [14]. The NMIS 2015 also clearly showed that a child's age, residence, and household wealth index are key factors in influencing the burden of malaria among under-fives in Nigeria [9]. Only fathers' educational level showed a significant relationship with Malaria prevalence in the study. The homogenous demography of the study subjects was a potential confounder here.

In this study, the sensitivity and specificity of RDT were 29.5% and 83.3% respectively, and for UMT, were 4.5% and 98.3% respectively. The PPV and NPV of RDT were 47.3% and 70.0% respectively while that of UMT was 57.1% and 67.1% respectively.

Oguonu., *et al*. in Enugu reported a UMT sensitivity of 83.75%, specificity of 83.48%, PPV of 77.91%, and NPV of 88.07% performing better than the findings in this study [4].

Oyibo., *et al*. in Lagos also reported a UMT sensitivity of 79%, specificity of 89%, PPV of 65%, and NPV of 94%. [2]. They pointed out that amongst < 5 years, UMT performed better with sensitivity (93%), and specificity (83%) respectively [5]. UMT performed very poorly in this study compared to what was reported by Oguonu., *et al*. and Oyibo., *et al*. [4,5].The lower parasitemia levels recorded in this study compared to what was reported by Oguonu., *et al*. and Oyibo., *et al*. might have been a major factor accounting for the reported outcome [4,5].

Oguonu., *et al*. and Okete., *et al*. both concluded that UMT should not be stand-alone for confirmation of malaria [2,4]. This was in keeping with the study's findings.

Since UMT performance was based on its ability to detect HRP-2 shed in urine, and given the strict exclusion of patients who had received Artemisinin combination therapy in the preceding five weeks, most of the patients may have been newly infected, and not secreting detectable quantities of the HRP-2 antigen in urine [15]. This reason may explain the poor performance of both RDT and UMT compared to microscopy in this study. Another possible reason could have been a variation between the methods used when testing with UMT amongst the researchers. While Okete., *et al*. and Oguonu., *et al*. allowed the strip to stand in the urine for 25 minutes (as recommended by the manufacturer) [2,4]. Oyibo, initially stood the UMT stripe in the urine for 10mins, before subsequently incubating it outside the urine for 15mins [4]. Likely, this might not have affected the outcomes in the expected way, as all the other studies interestingly recorded better UMT performance compared to this study.

The poor and in-homogenous performance of the RDTs was hypothesized to be possibly related to factors like storage conditions and kit quality regulations and standardizations [6].

RDT performed even more poorly in this study, compared to Wogu., *et al*. Garba., *et al*. and Nzekwe., *et al*. [16]. Nzekwe., *et al*. demonstrated that RDT performance varied significantly amongst different producers [17]. This also suggests wide variation in the product performance of RDT, which may be a result of product makeup and other factors like the ACT exposure status of the patients.

In this study, the false negatives for RDT were 70.5%, reflecting a high missed diagnosis. False positives amounted to 16.7% (29 enrollees), which could result in irrational drug use. The possible explanations, like kit-dependent performance variability factors and parasite density, have been earlier mentioned. A high false negative of 95.5% and a false positive of 42.9% for UMT were quite poor in this study. Parasite density affected UMT and RDT performance negatively.

Potential explanations for RDT false positivity include; Increase rheumatoid factor levels, toxoplasmosis, dengue, leishmaniasis, toxoplasmosis, and schistosomiasis [17-19]. False negativity for the HRP2-based test could be due to polymorphism of the HRP-2 molecule, gene deletions, prozone effects related to high parasite density, poor storage of the test kits, and poor transport situations (temperature and humidity) [20].

The mean parasite density in this study was 8,063.4 ± 60.9 parasites /µl. The range was 16 to 881,600 parasites/µl in this study. Oguonu., *et al*. in South-East Nigeria in a study of the general population reported a mean parasite density of 62,778.9 parasites/µl and a range of 60 to 792,600 parasites/µl [4]. These were higher than those obtained in this study.

The detection rate by the UMT increased with increasing parasite density in this study. Additionally, in this study, the sample with the least parasite count detected by the UMT had a parasite count of 17,720 parasites/µl.

UMT was only able to detect parasitemia in study subjects with parasite density of ≥ 5000 parasite/ul. This suggests that UMT detects the Plasmodium antigen in urine only with higher levels of parasitemia. This undermines its effectiveness in non-high transmission areas.

Ugah demonstrated the tendency of RDT performance to vary significantly as demonstrated above, meaning that the performance of RDT should be carefully observed whenever in use, and supported with microscopy if in doubt [21].

This research showed that only *Plasmodium falciparum* was isolated. This was in keeping with most studies in Nigeria, where the predominant specie *Plasmodium falciparum* [4,5,9].

Conclusion

The study revealed that RDT was inferior to microscopy in diagnostic performance. Its utility in clinical practice in our environment might be limited and it should be interpreted cautiously and confirmed with microscopy tests whenever possible.

The UMT, despite being the preferred test method amongst patient caregivers, displayed lower sensitivity and specificity compared to microscopy and RDT. It is therefore not recommended for use at present because of its low sensitivity and tendency to delay appropriate treatment due to high false negative results.

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Conflict of Interest

None that will have affected the outcome of the research.

Bibliography

1. Fed. Mins. of Health, National Guidelines for Diagnosis and Treatment of Malaria, 4th ed. NMEP, (2020).
2. J Okete., *et al*. “Reliability of Urine Malaria Test (UMT) for Malaria Diagnosis”. *AJRIMPS* 4.1 (2018): 1-9.
3. M Peter Asaga., *et al*. “Prevalence of Plasmodium Falciparum among Nigerians in Abuja and Central States: A Comparative Analysis of Sensitivity and Specificity Using Rapid Diagnostic Test and Microscopy as Tools in Management of Malaria”. *International Journal of Tropical Diseases* 2.1 (2019).
4. T Oguonu., *et al*. “The performance evaluation of a urine malaria test (UMT) kit for the diagnosis of malaria in individuals with fever in south-east Nigeria: cross-sectional analytical study”. *Malaria Journal* 13.1 (2014): 403.
5. W A Oyibo., *et al*. “Multicenter Pivotal Clinical Trial of Urine Malaria Test for Rapid Diagnosis of Plasmodium falciparum Malaria”. *Journal of Clinical Microbiology* 55.1 (2017): 253-263.
6. World Health Organization, Malaria microscopy quality assurance manual, Version 2. Geneva: World Health Organization (2016).
7. A Moody. “Rapid Diagnostic Tests for Malaria Parasites”. *Clinical Microbiology Reviews* 15.1 (2002): 66-78.
8. J Cunningham., *et al*. “A review of the WHO malaria rapid diagnostic test product testing programme (2008-2018): performance, procurement and policy”. *Malaria Journal* 18.1 (2019): 387.
9. Nigeria Malaria Indicator Survey 2015: Final Report. NMEP, (2016).
10. A G Samal., *et al*. “The sensitivity and specificity of a urine based Rapid Diagnostic Test for the diagnosis of *plasmodium falciparum* in a malaria endemic area in Odisha, India”. *Pathogens and Global Health* 111.7 (2017): 383-387.
11. J Chan., *et al*. “Patterns of protective associations differ for antibodies to *P. falciparum* -infected erythrocytes and merozoites in immunity against malaria in children”. *European Journal of Immunology* 47.12 (2017): 2124-2136.

12. D L Doolan., *et al*. "Acquired Immunity to Malaria". *Clinical Microbiology Reviews* 22.1 (2009): 13-36.

13. M Pinkevych., *et al*. "The Dynamics of Naturally Acquired Immunity to Plasmodium falciparum Infection". *PLOS Computational Biology* 8.10 (2012): e1002729.

14. E I Nwaneli., *et al*. "Malaria Prevalence and its Sociodemographic Determinants in Febrile Children- a Hospital-based Study in a Developing Community in South-east Nigeria.". *Journal of Preventive Medicine and Hygiene* (2020): E173.

15. U Dalrymple., *et al*. "How long do rapid diagnostic tests remain positive after anti-malarial treatment?". *Malaria Journal* 17.1 (2018): 228.

16. MN Wogu and F O Nduka. "Evaluating Malaria Prevalence Using Clinical Diagnosis Compared with Microscopy and Rapid Diagnostic Tests in a Tertiary Healthcare Facility in Rivers State, Nigeria". *Journal of Tropical Medicine* (2018): 1-4.

17. I T Nzekwe., *et al*. "Comparative evaluation of diagnostic accuracies of three rapid malaria testing kits".

18. J Iqbal., *et al*. "*Plasmodium falciparum* Histidine-Rich Protein 2-Based Immunocapture Diagnostic Assay for Malaria: Cross-Reactivity with Rheumatoid Factors". *Journal of Clinical Microbiology* 38.3 (2000): 1184-1186.

19. P Gillet., *et al*. "False Positivity of Non-Targeted Infections in Malaria Rapid Diagnostic Tests: The Case of Human African Trypanosomiasis". *PLOS Neglected Tropical Diseases* 7.4 (2013): e2180.

20. P Pati., *et al*. "High proportions of pfhrp2 gene deletion and performance of HRP2-based rapid diagnostic test in Plasmodium falciparum field isolates of Odisha". *Malaria Journal* 17.1 (2018): 394.

21. U I Ugah., *et al*. "Evaluation of the utility value of three diagnostic methods in the detection of malaria parasites in endemic area". *Malaria Journal* 16.1 (2017): 189.