

## **Comparative Study of Rapid Antigen Detection Assay and Simple Microscopy in the diagnosis of malaria**

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**Abstract:** *One of the greatest challenges faced by many underdeveloped countries in the quest for malaria control is limited access to proper and effective diagnosis. This has been compounded by wars, population movement, endemicity of the infection and drug resistance. Although microscopy has remained the gold standard for the diagnosis of this infection, it is costly, time and labour intensive and requires great expertise. This study was conducted to compare the performance of the gold standard microscopy with the Pf-HRP2 rapid diagnostic tests with the view of the later serving as alternative to the former especially in endemic areas where experts microscopists are lacking. A total of 100 individuals were enrolled in this study. Out of this 23(23%) persons tested positive with HRP-2 Antigen Detection Assay while 53 (53%) persons tested positive with microscopy method. This showed that 30 false negative results were recorded by the Pf-HRP2 antigen detection tests. The prevalence of infection by age distribution with both methods of diagnosis was lower in younger age groups than in the older ones. In both methods also, infection was higher in females than in males. This study shows that microscopy is more reliable and sensitive for the routine diagnosis of malaria than HRP-2 antigen detection assay. Thus it still remains the gold standard for malaria diagnosis. It is therefore recommended that scientists/lab technicians should rely more on microscopy.*

**Keywords:** *malaria, HRP-2, microscopy, diagnosis, antigen.*

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### **I. Introduction**

Malaria is a life threatening parasitic disease transmitted by female Anopheles mosquitoes. It is transmitted in 108 countries containing three billion people and causes nearly one million deaths each year (Bhat et al, 2012). Four of the known species of Plasmodium that commonly infect man are Plasmodium falciparum, P. ovale, P. vivax and P. malariae. Symptoms include chills, high fever, rigors and flu-like illness. Malaria is often misdiagnosed because of these common symptoms. It is the most highly prevalent tropical disease, with high morbidity and mortality and high economic and social impact (WHO, 2001).

Malaria together with HIV/ AIDS and tuberculosis, is one of the world's most vital public health challenges compromising development in poverty stricken countries and accounting for up to an overwhelming 2.7 million death per annum. More than 3 billion people ( $\cong$ 40%) reside in areas of the world where it is prevalent (WHO, ). As such, the disease is largely responsible for the poor economic growth of these areas; which further contributes to more cases of malaria (Korenromp et al., 2005). It has remained a major public health problem in Nigeria and is responsible for 30% childhood and 11% maternal mortality (FMOH, 2005).

Malaria is a complicated disease and its spread may be attributable to variety of factors such as ecological and socio- economic conditions, displacement of large population groups, agricultural malpractices causing an increase in vector breeding, parasite resistance to anti malarial drugs and vector resistance to insecticides.

Early diagnosis and prompt treatment of malaria is the key to minimizing its mortality and morbidity but the diagnostic capabilities and diverse clinical presentations remain a challenge for the laboratories and hindrance for effective malaria control. Therefore many victims of malaria die because the disease is either not diagnosed in time or was inappropriately carried out by health workers. The quest for newer and easier diagnostic methods for malaria has picked up momentum in the past decades. The urgency and importance of obtaining results quickly from examination of blood samples from patients with suspected acute malaria render some of the more sensitive methods for malaria diagnosis impractical for routine laboratory use. Endemic malaria, population movements and foreign travel all contribute to the malaria diagnostic problems faced by the laboratory that may not have appropriate microscopy expertise available (Moody, 2002). Laboratory confirmation of malaria infection requires the availability of rapid and specific test at an affordable cost. In attempt to achieve this, new tests have been developed but conventional method by microscopy remains the gold standard against which all other tests have been evaluated (WHO, 2004). Though microscopy is considered to be the gold standard for malaria diagnosis, many health facilities in sub-Saharan Africa lack properly functioning microscope, quality control systems and well-trained laboratory technicians (Allen et al., 2011). Other factors that lead to poor microscopy includes slide preparation techniques, workload, population

movements and foreign travels. As a result, only a small proportion of all malaria diagnoses in Nigeria are based on microscopy while others are based on clinical signs and symptoms.

Rapid Antigen Detection Assay is a device that detects malaria antigen in a small amount of blood by immunochromatographic assay with monoclonal antibodies directed against the target parasite antigen and impregnated on a strip. They are simple, easy to perform and interpret, give quick results and require no capital investment or electricity (Bhat et al., 2012). However, there are some setbacks associated with the use of RDTs in the diagnosis of malaria. There are expensive, associated with false positive result due to persistent antigenemia and cross-reactions with auto antibodies such as rheumatoid factor and false negative results in severe malaria due to immune-complex formation, prozone phenomenon and unknown cases (Mendiratta et al., 2006).

Due to genetic variations and drug resistance, many accepted morphological appearances of the causative agent of malaria are constantly altered and this affects accurate diagnosis of the infection. It therefore becomes imperative that the different diagnostic techniques, their efficiency and comparison be evaluated from time to time. In this study, we have made effort to compare between the peripheral blood smear examination and Histidine Rich Protein-II antigen detection tests in asymptomatic malaria.

## **II. Materials And Methods**

### **Study Area**

This study was carried out at Ekpelu community in Ikwo Local Government Area of Ebonyi State. The community is located at the extreme part of Ikwo North having common boundary with Ezza South Local Government Area. Other communities that have common boundaries with the community are Ameka, ekpaomaka and Ekka-Awoke communities all in Ikwo Local Government Area. Her population is encouraging though a bit underdeveloped with poor distribution of basic amenities such as electricity, pipe borne water, good roads, primary and secondary health centre and poor communication network services. They are some seasonal rivers, streams and pounds scattered all over the area. The climate is tropical and the vegetation characteristics are predominantly the rainforest at an average rainfall of about 1300mm and average atmospheric temperature of about 28-30<sup>0</sup>C.

The soil type is partly swamp and partly upland that is rich in fertility. Due to that, the inhabitants of the area are mostly farmers. Due to poor road network, their goods are mostly sold and bought within the domestic markets therein. Their major farm products include yam, rice, cassava, potatoes, vegetables.

### **Study Population**

The study population comprised of individuals of all ages. Both males and females were included in the study.

### **Ethical Consideration**

Before the commencement of the study, informed consent was obtained from the community head and the head of each family involved.

**Sample Collection:** Careful procedures were adopted in the collection of blood samples by swabbing the area to be punctured with 70% alcohol and allowing to dry before collection. One hundred individuals were used for the study. Leishman stained thick and thin smears and Histidine-rich protein 2 of Plasmodium falciparum (PfHRP2) antigen detection using paracheck Pf dipstick were performed.

**Peripheral smear preparation:** Thick and thin smears were prepared on clean grease-free slides, dried and stained using standard guidelines as described by (Cheesbrough, 2005). After staining, the smears were examined under the microscope using x100 objectives with oil immersion (WHO, 1991). At least 100-200 microscopic fields were examined before results were recorded in the thick smears. To determine the species of the parasite, the red blood cells in the tail end of the thin smear were examined.

**Histidine-rich protein 2(HRP-2):** This was done using paracheck pf dipstick according to manufacturer's instruction manual. It is a rapid self performing quantitative immunochromatographic test for the detection of P. falciparum specific HRP-2 antigen in whole blood. The kits were all of the same batch and were used before the expiry dates

**Statistical analysis:** Differences between study parameters were tested by Chi-square tests and 95% confidence interval was computed to find the significant features.

### III. Results

Of a total of 100 persons examined, 76% (76 out of 100) of the individuals were positive for malaria parasite and all the cases were *Plasmodium falciparum*. Out of this number of positive cases, 53% (53 out of 100) cases were positive by conventional thick smear method of examination while 23% (23 out of 100) were positive by HRP- 2 antigen detection method. Among the age ranges, individuals within 0-6 years recorded the highest infection 12(38.71%) and 20 (64.52%) in RDTs and Microscopy respectively. This was followed by 7-12 years which recorded 5(27.78%) and 12(66.67%) while those within 37 and above years recorded the least (00.00) and 02(40%). In both RDTs and microscopy, infection occurred more in females 15(25%) than the males 36 (60%).

**Table 1: Malaria infection due to age using RDTs and Simple Microscopy**

Age Range (Years)	No Examined	No infected (%)	
		RDTs	MICROSCOPY
0-6	31	12(38.71%)	20(64.52%)
7-12	18	5(27.78%)	12(66.67%)
13-18	18	2(11.11%)	07(38.89%)
19-24	09	1(11.11%)	4(44.44%)
25-30	13	1(7.69%)	6(46.15%)
31-36	6	2(33.33%)	02(30.33%)
37-above	5	00(00%0	02(40%)
<b>Total</b>	<b>100</b>	<b>23(23%)</b>	<b>53(53%)</b>

**Table 2: Malaria Infection among the sexes Using RDTs and Microscopy**

Sex	No Examined	No Infected (%)	
		RDTs	MICROSCOPY
Male	40	08(20%)	17(42.50%)
Female	60	15(25%)	36(60%0
<b>Total</b>	<b>100</b>	<b>23(23%)</b>	<b>53(53%)</b>

### IV. Discussion

Malaria is a life threatening parasitic disease transmitted by female *Anopheles* mosquitoes. It is a highly prevalent tropical disease, with high morbidity and mortality and high economic and social impact (WHO, 2001). Its occurrence is confirmed by the detection and identification of trophozoite stage (ring form) in blood sample examined on microscope and the reaction of antigen and antibody in the Rapid Antigen Detection Assay (RDTs). Prompt and accurate diagnosis of malaria is a key to effective management and of malaria because it helps in early start of appropriate antimalarial drug to prevent complications (Bhat et al., 2012). In view of this, new rapid diagnostic techniques have been developed and evaluated in recent years.

In the present study, the prevalence of malaria in a rural community in Ebonyi State, Nigeria was assessed using simple microscopic and Histidine-rich protein 2 (HRP-2) methods. The Rapid Antigen Detection Assay results indicated a prevalence of 23 (23%) while microscopy indicated the total prevalence of 53 (53%) out of the 100 persons that were examined. However, the result in both cases are lower than 72% recorded by Adefioye et al., 2007 at Oshogbo in western Nigeria. This may be attributed to the fact that the present study was carried out during dry season when stagnant water bodies have been dried up and the breeding sites of mosquitoes interrupted or progress in the control of malaria through the use of insecticide treated mosquito nets by the residents.

The result clearly indicated that HRP- 2 antigen detection test had lower sensitivity compared to microscopic analysis method. Thus it recorded false negative cases. This is in agreement with other reports from different settings (Moody, 2002, Singh et al., 2013). Since most of the individuals have asymptomatic infection, the level of parasitaemia might have been low. Hence, HRP- 2 antigen detection test failed to detect the parasites because its sensitivity decreases at low levels of parasitaemia (Bhat et al., 2012). However, it will be of particular use in rapid diagnosis of febrile patients. Other explanations could be defects in the device (Bell et al., 2006), failure of the parasites to express the antigen due to deletion of the gene *pfhrp2* (Koita et al., 2012), anti Pf-HRP2 antibodies in humans (Biswas et al., 2005) or storage conditions (WHO, 2001). Therefore, microscopic examination of blood utilizing blood film is still regarded as the mainstay of malaria diagnosis (Krafts et al., 2011).

The prevalence of malaria parasite by both methods showed that, age group 0-6 years had the greatest risk of infection followed by 7-12 years (See Table 1). This corroborated Ani, 2004, and Nwaorgu and Orjiaka (2011) who all reported decline in prevalence by age. This showed that the younger children are more prone to malaria infection than other older ones and that age could be an important factor infection. Malaria being a protozoan infection induces immunity to re-infection. Therefore the older members of the population may have acquired immunity to the infection. Another reason could be shortage of insecticides treated nets in some

families. Most children therefore do not sleep under the nets. This is due to over population in household, inadequate supply of insecticide treated mosquito nets by government or inability of the parents to buy treated insecticide net due to poverty

The prevalence of malaria parasite due to sex in both methods was higher in females than in males (See Table 2). This sex distribution showed that females are more prone to the infection which is in accordance with the previous study of Adefioye et al, (2007). This however, contrasts Nwaorgu and Orajiaka (2011) and Okafor and Oko-Ose, (2012) who reported higher prevalence in males in different parts of the country.

## V. Conclusion And Recommendations

There is high prevalence of malaria infection in this rural Community which presents a need for intensified control programmes in the area. There was decline in prevalence as age increased and infection was higher in females than males. Rapid antigen detection kits are useful in field studies especially in rural and endemic areas but its sensitivity and specificity is low at low parasitaemia. In evaluating or reviewing the methods for malaria diagnosis, sensitivity, rapidity availability and cost are taken into consideration. Microscopy meets this requirements and remains the gold standard method for malaria diagnosis.

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