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# Comparison of the Accuracy and Efficacy of First Response and Standard Diagnostics for the Rapid Diagnosis of *Plasmodum falciparum* in the Federal Capital Territory of Nigeria.

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ABSTRACT: The study was carried out to compare two rapid diagnostic test kits (Standard Diagnostics (SD) and First Response) which are often used locally for the diagnosis of *P. falciparum*. 300 patients participated in the study out of which 162(54%) were females and 138(46%) were males.109 samples (36.3%) of the total samples were positive for *P. falciparum* and 191(63%) negative with microscopy .67 samples (22.3%) were positive and 233(77.7%) negative with SD while 63(21%) and 237(79%) were negative with First Response. The study shows that the sensitivity of SD is 61.5% with specificity of 100% while First Response has 57.8% sensitivity and 100% specificity when compared with thick blood film Microscopy performed by an expert microscopist. Both sensitivity and positive predictive value of SD prove it to be better than first Response. Parasite density as low as 40/µl was detected by Microscopy. Hence Standard Diagnostics (SD) is better than First Response RDT in the rapid diagnosis of *P. falciparum* though microscopy still remain the gold standard.

## Introduction

Malaria is adjudged a major public health problem in Nigeria where it is endemic especially in rural populations as is the case elsewhere in Africa (1), and is one of the leading causes of morbidity and mortality worldwide, causing about 3,000 deaths per day and killing a child every 30 sec (2). Early and proper diagnosis of malaria is the first step in treatment and control of the disease. Various diagnostic methods have been developed over the years including the recent method – Rapid Diagnostic Tests (RDTs) which are antigen -antibody tests targeting either the histamine-rich protein -2 of *P. falciparium* or a parasite-specific lactate dehydrogenase (3)

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Microscopy has been the age long golden method of diagnosing malaria parasites. The associated problems with microscopy led to the development of other faster methods like polymer chain reaction (PCR) and Rapid Diagnostic Tests (RDTs), even though they are more costly. These additional diagnostic options are to be reviewed and compared to more traditional method. This research is centered on reviewing and comparing two kits of one of these methods to determine their efficacy and accuracy.

Worldwide results of different RDTs have deferred a lot, with some reporting higher sensitivities and specificities while others reporting the opposite (4,5). The availability and cost of RDTs are among the factors that determine which RDTs will be used in a particular setting. There is need, therefore, to evaluate the efficacy and accuracy of some available RDT kits in the markets that are used to diagnose malaria locally. This direct product comparison will assist the policy makers and programe managers in taking procurement decision which will invariably encourage improvement in the quality of manufacturing for better and efficient diagnosis of malaria.

## Materials and Methods

### Study Area

This study was carried out in the FCT, Abuja. Two hospitals, (Federal Staff Hospital, Garki II and Federal Staff Hospital Annex1) among symptomatic patients visiting the hospitals for treatment. Federal Staff Hospital Garki II is located along Akintola Boulevard Street in Garki II while Federal Staff Hospital Annex 1 is located on ground floor Federal Secretariat complex Phase 1 along Shehu Shagari way in Central area both in Abuja – F.C.T. Federal Staff Hospitals are owned and managed by Federal Ministry of Health.

### Study Population

Symptomatic patients attending hospital for treatment were investigated. There was no age or sex barrier. 300 blood samples were examined using the two RDT kits-SD and First response. The samples were also examined microscopically for malaria parasites and the density of parasites/µl of blood was estimated.

### Sample collection

Venous blood was collected into sample bottle containing potassium EDTA anticoagulant. Using a 5ml syringe and needle the vein was punctured and about 4ml of blood drawn into a labeled EDTA anticoagulant bottle and mixed gently

### Preparation of Blood Films.

Both thick and thin films were made from each patients sample immediately at the point of collection on same slide as described by Chesbrough (6).

### Procedure for Preparation of Thick and thin Blood Films

A small drop of blood (2µl) was placed at the centre of a clean, grease free microscope slide, for thin film and a larger one(6µl) about 15mm to the right for thick film. Immediately the thin film was spread using a smooth edged glass spreader. Without delay the larger drop of blood was spread to make a thick smear covering an area of about 15mm. The films were allowed to air dry in a horizontal position on a flat surface.

The thin film was fixed with absolute methanol for 2 minutes to ensure that the smear does not wash away and to fix the cells well and the thick film was heat fixed in a hot air oven at 40°C for 20 mins.

**Staining of Films using Giemsa Stain.**

10% (1:10) dilution of the Giemsa stain was made in buffered water (pH 7.1-7.2) immediately before staining and the films were stained using the following procedure as stated by WHO, (7).
The slides were placed on a staining rack and flooded with 10% Giemsa stain and stained for 10minutes then washed gently with clean water, the back wiped and air dried.

**Estimating Parasitaemia/µL of Blood from the Thick Film.**

Counting of Parasite density in blood in a micro liter (µl) of blood was done using WHO method of counting parasite density in thick film (8). The number of parasites counted alongside the count of 8000 leukocytes (WBC) is equivalent to the number of parasites in one micro liter of blood.

**Detection of Malaria Parasite in Whole Blood Using First Response and SD Rapid Diagnostic Test Kits**

**Procedure for Performing SD Rapid Diagnostic Test for Detection of Malaria**

Each of the sample examined microscopically in which the malaria parasite density was estimated was also tested for the detection of malaria antigen using both First Response and SD kits. The tests were done the same day of sample collection, and according to the manufacturer's guidelines and procedures. The test kit was allowed to assume room temperature. The cassettes were brought out from the foil and placed on a flat, dry surface. 5µl of blood was placed in the sample well and 4 drop of assay diluents into the diluents well. The results were read after 15 minutes' but not more than 30 minutes.

**Procedure for Performing First Response Rapid Test for Malaria Detection**

After allowing the test kit to assume room temperature, the test is performed as follows; 5µl of blood was added into the sample well on the device.
2drops of sample diluents was added in the diluents well. The result was read after 20 minutes but not exceeding 30 minutes.

**Statistical Analysis**

The data were analyzed using the statistical software *Epi info.*

## Results

**Sex Related Distribution of Study Participants**

A total of 300 patients were tested for malaria parasites using the Standard Diagnostics (SD) rapid diagnostic test and First Response rapid diagnostics test, and the results compared with microscopy (Gold standard). Figure 1 showed that out of this number a greater number of 162 (54%) were females and 138 (46%) were males, giving a male to female ratio of 1:1.2

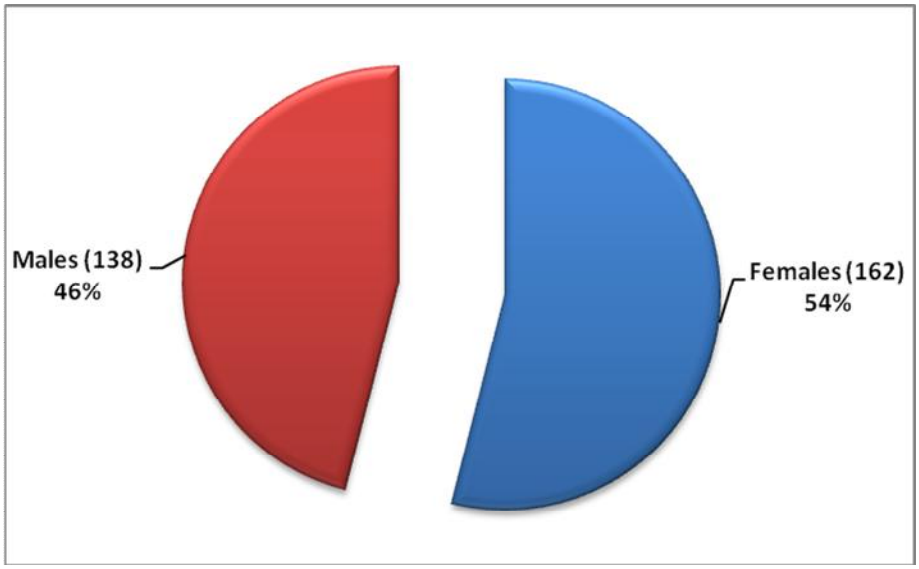


**Figure 1 : Sex Related Distribution of Study Participants.**

**Age Related Distribution Of Study Participants**

Figure 2 presents the age related distribution of study participants. This figure shows that (208) participants corresponding to 69.3% are more than 25 years of age, while (92) represented only 30.7% are below 26 years of age.

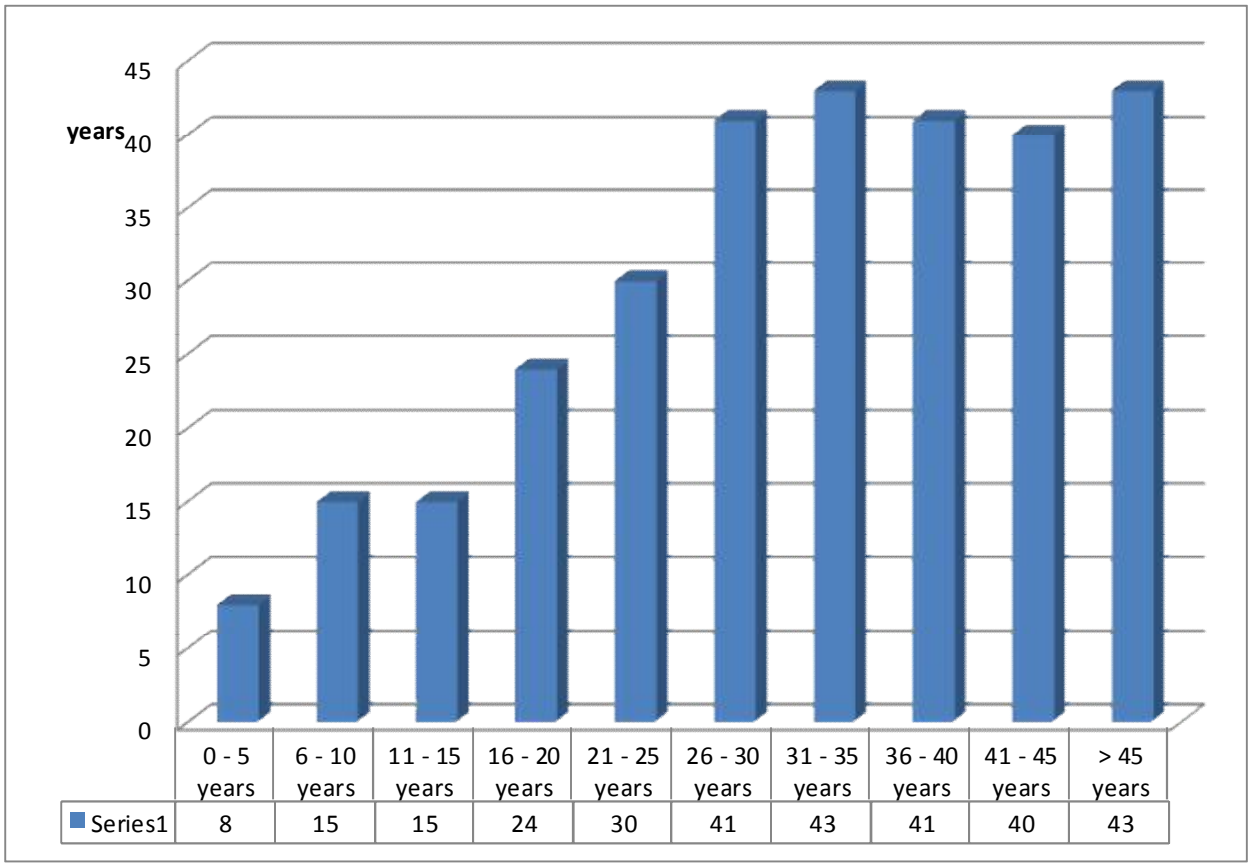


**Figure 2: Age related distributed of study participants**

Table 1 shows that of the 300 samples collected, 109 (36.3%) were positive with microscopy and 191 (63.7%) were negative. 63(21%) and 67 (22.3%) were positive with first response and standard diagnostics (SD) rapid test kits respectively leaving negative results of 237(79%) and 233 (77.7%) respectively.

**Table 1:Performance of microscopy, Standard Diagnostics and First Response in the diagnosis of *P. falciparum***

| Laboratory result | Microscopy (n=300) | First response (n=300) | SD (n=300) |
|---|---|---|---|
| Positive | 109 (36.3%) | 63 (21%) | 67 (22.3%) |
| Negative | 191 (63.7%) | 237 (79%) | 233 (77.7%) |
| **Total** | **300** | **300** | **300** |

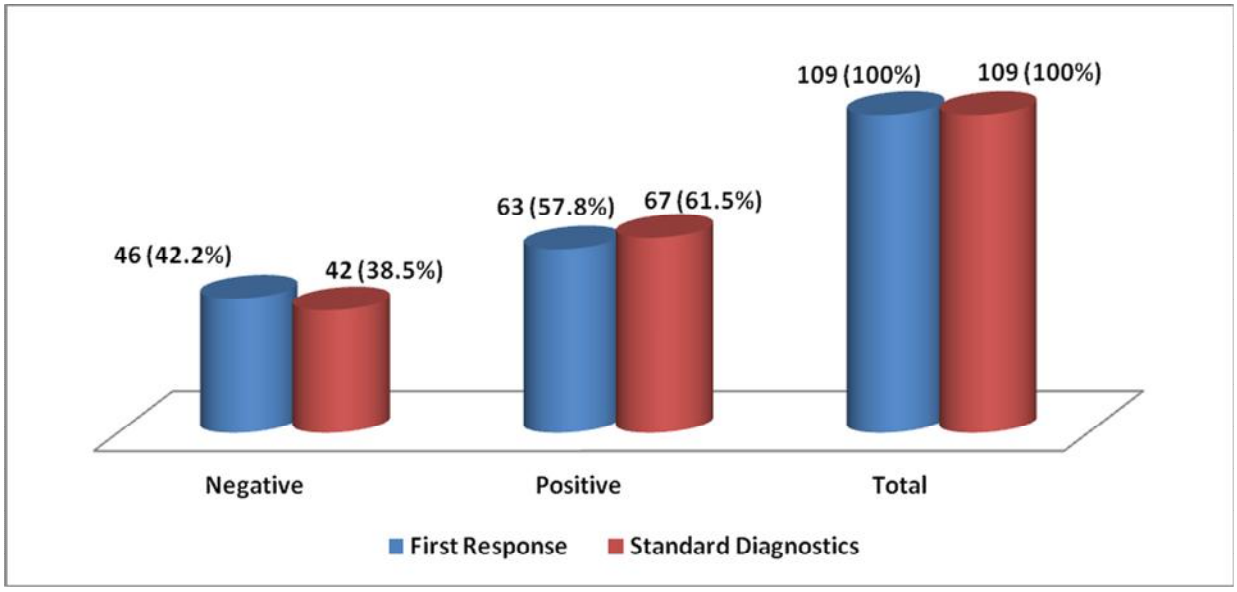


**Figure 3: Diagnostic sensitivity of First Response rapid test kit and standard Diagnostics rapid test kit relative to blood microscopy in detecting *Plasmodium falciparum***

Figure 4 shows that the diagnostic specificity of First Response rapid test kit and Standard Diagnostics rapid test kit were similar (100%) relative to blood microscopy. All the samples that tested negative with microscopy also tested negative with both SD and First Response rapid test kits proving both to be equally specific for diagnosis of *Plasmodium falciparum*.

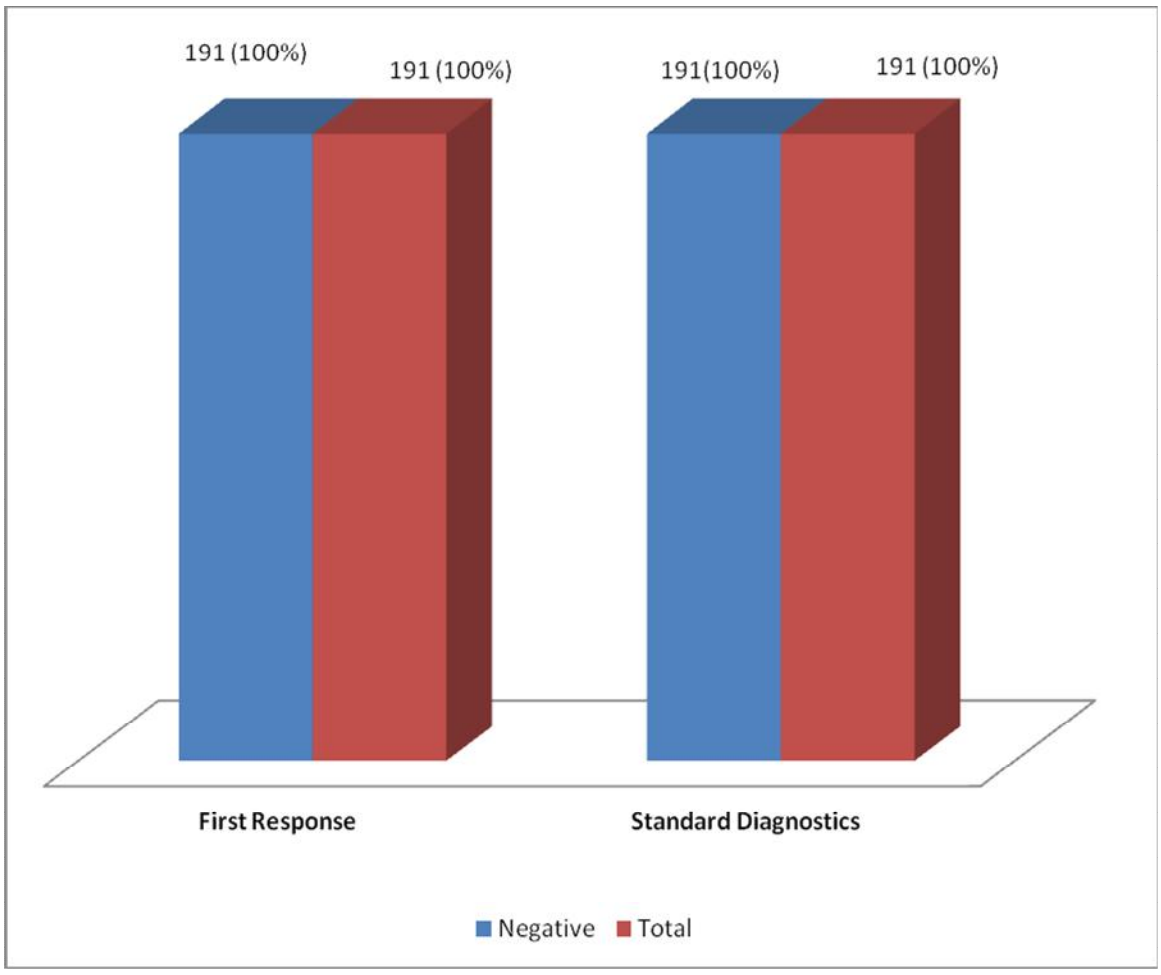


Figure 4: **Diagnostic specificity of First Response rapid test kit and Standard Diagnostics rapid test kit relative to blood microscopy in the laboratory diagnosis of *Plasmodium falciparum***

Figure 5 shows the measure of agreement between the two rapid diagnostic kits. Before the point of the agreement, the curve of SD rapid test kit is above that of First response giving SD a better predictive value than First Response in low density parasitamia.

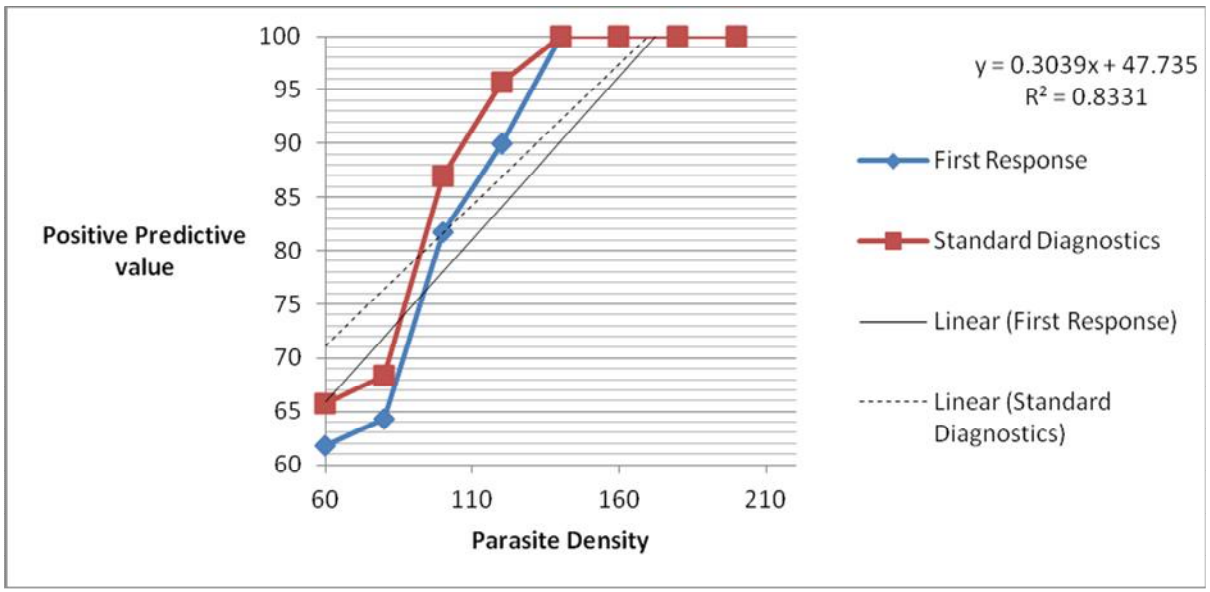


**Figure 5. Positive predictive values of First Response rapid test kit and Standard Diagnostics rapid test kit relative to malaria parasite density using microscopy**

## Discussion

The two RDTs compared – Standard Diagnostics (SD) and first Response both detect the Histamine Rich protein 2 which is expressed only by P. *falciparum*. Thus only samples which had P. *falciparum* were tested. In this work while SD is having a sensitivity of 61.5%, first Response has that of 57.8%. This does not agree with the work (9) who compared 5 RDT kits including first Response and found out that first Response had a sensitivity of 95.8%.

Dietz *et al* (1995) recorded a sensitivity and specificity of 77 – 98% and 83- 98% respectively with Parasight F, a RDT which also detects the HRP2 as SD and First Response. Their work also differ slight with this in which SD has a sensitivity and specificity of 61.5% and 100% while first Response has that of 57.8% and 100%.

However, previous works on various RDTs showed differing sensitivity and specificity. (4) Noted that worldwide results of different RDTs have differed a lot, with some reporting higher sensitivities and specificities while others report the opposite.

A lot of reasons could be responsible for these disparities in sensitivity and specificity. Temperature variations especially in the tropics could lead to deterioration of the reagents embedded in the kits as well as mode of storage and storage facilities could also contribute to this disparity. (11) Suggested that among the reasons for disparity in sensitivity and specificity of RDT is in different epidemiological setting as in Para HIT with low sensitivity and specificity in Tanzania and high sensitivity and specificity in India.

Results from this research also indicated among reasons for this disparity is the parasite density. Most of the samples with parasite density of 120 parasites/µl tested negative with Standard Diagnostics and First Response while all the samples that had parasite density <120µl tested negative to both kits. This findings agrees with the work of (12) who found the sensitivity of immunochromatogaphic test for HRP-2 for the detection of *P. falciparum* in blood samples to be 77-98% when more than 100 parasites/µl are present and (13) who stated that a negative test was adequate to exclude parasitemia of <300 parasites/µl and in some instances an even higher parasitemia.

Furthermore, our findings are in consonance with that of (13) who opined that test band intensity in the RDTs correlated with parasite density in microscopy.

The results of the thick blood film microscopy detected parasite density as low as 40 parasites/ul of blood and this is in agreement with (14).

In conclusion, the Sensitivity of SD (61.5%) is higher than that of first Response 57.8%. The positive predictive value of SD is consistently superior to First Response below the parasite density of 140/µl. With the sensitivity of SD being higher and positive predictive value being superior to that of First Response, SD is obviously a better RDT for the diagnosis of *P. falciparum.* In high density Parasitaemia both kits have similar positive predictive value (100%). The specificity of both SD and First Response are similar in the diagnosis of *P. falciparum*.

Finally, Microscopy remains the gold standard when it is done by high skill manpower since it has detected parasites as low as 40 parasites/µl of blood.

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