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Evaluation of the efficacy of rapid diagnostic tests compared to microscopy in the diagnosis of malaria infection

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Abstract
Malaria controls involve prompt diagnosis of malaria parasite by expert microscopy, Rapid Diagnostic Tests (RDTs), or any other available tools. This study was aimed at comparing the efficiency and effectiveness of microscopy and rapid RDTs in the detection of malaria parasite. The study was conducted on patients who were directed to the parasitology laboratory department for blood screening for malaria parasites at specialist hospital, Jimeta, Adamawa state. Two hundred and sixty-two (262) blood samples were collected and screened using both microscopy and RDTs according to the manufacture's guidelines. The result of the study revealed high prevalence in patients within 5 – 20 years of with 85(61.15%) and 83(59.71%) for microscopy and RDTs respectively. Out of 262 participants enrolled in this study 138(52.67%) were males and 124(47.33%) females. Microscopy was found to be positive in 170(64.89%) while RDTs was positive in 159(60.69%). Microscopy revealed sensitivity, specificity, positive predictive value and negative predictive value of 100%. However, RDT revealed sensitivity, specificity, positive predictive value and negative predictive value of 93.29%, 93.81%, 96.23% and 89.22% respectively. This study revealed that microscopy remains the gold standard method for malaria diagnosis especially when there are expert in microscopy. Furthermore, in areas when expert in microscopy is not available, RDTs can be used for quick diagnosis of malaria parasites.

Keywords: Evaluation, efficacy, rapid, diagnostic, microscopy, malaria, infection

1. Introduction
Malaria is caused by different species of *Plasmodium* parasite which are found in human and non-human. *Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae* and *Plasmodium ovale* are found in humans while *Plasmodium knowlesi, Plasmodium berghei* and *Plasmodium vinkei* are found in non-human. *Plasmodium falciparum* and *Plasmodium vivax* are widely spread and they are mostly found to be associated with cases of mixed- species of malaria, also known as co-infection with more than one species or genotype of *Plasmodium* [1].
Malaria is a disease cause by bite of infected anopheles mosquito that harbored plasmodium parasites; the *plasmodium* parasite is transmitted through the blood stream. It is a life threatening disease and has continued to pose a threat to the public health sector. Millions of people were affected globally but it mostly affects peoples in Africa, Asia and South America. According to world health organization report of 2015, malaria is endemic in over 100 countries with approximately three million people at risk of infection. In 2009 there are 225 million cases of infections which lead to approximately 781000 deaths [2]. The disease has remained a major public health concern in Nigeria because the disease is responsible for 13% child and 11% maternal mortality. Malaria accounts for 300,000 deaths each year and about 60% of outpatient visit [2].
In developing countries, microscopy the method that mostly employed in the diagnosis of malaria infection. Hence, microscopy is regarded as the gold standard for laboratory diagnosis of malaria in many developing countries. Expertise in microscopy may be lacking in most endemic areas in the developing countries [1]. However, in a situation where expert microscopy is lacking, rapid diagnostic test (RDT) can serve as a useful alternative to microscopy [3].

Rapid diagnostic test plays a key role in malaria diagnosis especially in areas were expert microscopy is lacking. They are used in elimination control programs to avoid unnecessary anti-malaria therapy and preventing the emergence of drug resistant [4]. The microscopy is less advantageous to rapid diagnostic test because RDTs provide quick and reliable results and less skilled persons are required in malaria diagnosis. Furthermore, RDTs do not require electricity or any equipment [1].

This study was design to find a better way of easy, fast and reliable malaria diagnostic technique. The study aimed in finding an alternative convenient and effective away to diagnose malaria especially in rural areas. The finding of this study will be useful in routine diagnosis of malaria in endemic (rural) areas where microscopy expertise are very limited since RDTs are faster and easy to use by both trained and untrained personnel's. The study evaluates the efficacy of RDT (HRP-2 and pLDH/HRP-2) compared to microscopy in the diagnosis of malaria.

2. Materials and Methods

2.1 Ethical Clearance

Ethical clearance was obtained from the ethical review committee of Jimeta Specialist Hospital, Adamawa State. Inform consent of the patient's parents and/or guardians was taking before the samples were collected for this study.

2.2 Study Design

The study was conducted between July and August to assess the performance of rapid diagnostic test (RDTs) and standard microscopy. The study participants were enrolled were through the specialist hospital Jimeta. Patients presented to the specialist hospital Jimeta with suspicion of malaria irrespective of their age and gender were included in the study; after taking written informed consent. The study design was in compliance with the standard methodological guidelines for presentation of diagnostic studies [5].

2.3 Sample Collection

Two hundred and sixty two (262) blood samples were collected for this study and 2mL of blood samples was collected screened using RDTs and microscopy. All RDTs and blood films for a specific blood specimen were given identical codes for later comparison of the result.

2.4 Thin and Thick Film Preparation

Two mL (2mL) of venous blood was collected from each of the study participant. A drop of the blood was placed on a slide; a spreader with a smooth edge was used to make a short thin film at about 45 degree. Thick films were also made by using a corner of spreader, spreading the larger drop of the blood to the correct thickness to form a square at about 1.5cm. The films were allowed to air dry while protected from flies, ant and dust. Each slide was labeled with patient identity number using a black lead pencil [5].

2.5 Determination of Malaria Parasite by Microscopy

Duplicate thin and thick slides films were made from all the blood samples, and stained using 5% Giemsa stain for 10 minute. Determination of malaria by microscopy was performed by two experience (well trained) microscopist for identification of the malaria parasite species.

The number of parasite was counted against 200 leucocytes and quantification of parasite density was estimated by assuming 800 leucocytes/μL of blood [6]. Samples were considered negative when no parasite is detected. However, to check for inter observer difference a double- blinded cross reading of a random 50 blood slide was performed by another microscopist [6].

2.6 Determination of Malaria Parasite by RDTs

The RDT test was performed according to the manufacturer's recommended procedures [7]. The RDT cassettes were labeled with the patient number. According to the manufacture's instruction, a drop of the blood was transferred in to the sample well marked (s) on the cassette followed by addition of assay buffer to the blood in the sample well containing the blood. The blood- buffer mixture was allowed to run across the test and control window. Result was read after 15-20 minute [6].

2.7 Determination of Sensitivity of RDTs

The sensitivity of the RDTs was determined by taking the percentage of positive malaria test by RDTs from the total number of malaria positive samples by microscopy [6]. The sensitivity was calculated using the formula below;

$$\text{Sensitivity} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Negative}} \text{ x } 100$$

Sensitivity was defined as the probability that a truly infected individual will test positive.

2.8 Determination of Specificity of RDTs

The specificity of the RDTs was determined by taking the percentage of malaria negative test by RDTs from the total number of malaria negative samples by microscopy [8]. The specificity was calculated using the formula below:

$$\text{Specificity} = \frac{\text{True Negative}}{\text{True Negative} + \text{False Positive}} \text{ x } 100$$

Specificity was defined as the probability that truly un-infected individual will be tested negative [5].

The Positive Predictive Value is the probability that those individuals testing positive by rapid diagnostic test (RDT) were truly infected.

$$\text{Positive Predictive Value} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Positive}} \text{ x } 100$$

The Negative Predictive Value is the probability that those individuals testing negative by rapid diagnostic test (RDT) were truly uninfected.

$$\text{Negative Predictive Value} = \frac{\text{True Negative}}{\text{True Negative} + \text{False Negative}} \text{ x } 100$$

Where TP = true positive, FP = false positive, TN = true negative, and FN = false negative.

2.9 Plasmodium, Species Identification

Method described by Lee *et al.,* (2006) for species identified on the basis of their cell morphology using microscopy was employed [5].

2.10 Data Analysis

The Pearson Chi-square test was used to determine significance of results and p. value < 0.05 was considered statistically significant [5]. Sensitivity, specificity, positive predictive value and negative predictive values were calculated by comparing with the standard of 100% hypothetical sensitivity, specificity, and positive and negative predictive values.

3. Results and Discussion

The study participants were categorized among the following age groups 0-5yrs, 5-20 years and 20- above; the result of the microscopy showed that 32, 85 and 53 were positive for the age groups respectively. The results of the study reveals that people within the age of 5-20 years have the highest malaria prevalence with 85 positive cases while those within the age of 0-5years have the lowest number of malaria with 35 positive cases. However, he result of age distribution of malaria parasite by standard rapid diagnostic (RDT) test showed that the malaria parasite case is higher among individual that are within the age bracket of 5-20 years with 83 positive cases and also lowest in those within 0-5 years with 25 positive malaria cases out of the 159 positive cases as described in Table 1.

Table 1: Age Distribution of Malaria Parasite by Microscopy and RDTs

	Microscopy		RDT	
Age	**Positive Samples**	**Negative Samples**	**Positive Samples**	**Negative Samples**
0-5yrs	32(80.00%)	8(20.00%)	25(62.50%)	15(37.50%)
5-20yrs	85(61.15%)	54(38.85%)	83(59.71%)	56(40.29%)
20-above	53(64.63%)	29(35.37%)	51(62.19%)	31(37.81%)

Routine malaria diagnosis is focused on detection of asexual parasite stage in the stained blood sample using microscopy or detection of parasite antigen using RDTs. This study evaluated the performance of microscopy and RDT in the diagnosis of malaria. The prevalence of malaria is higher in children within the age of 0 – 5 years. Higher percentage was found in children the study participants within the age of 0-5years in both microscopy and RDT. This is because their immunity to malaria has not yet developed. In 2006, Garba *et al.,* reported higher positivity rate of malaria in children of 5years old [9].

Table 2 revealed the result of the distribution of malaria parasite by microscopy and RDT in relation to sex of study participant. The study result depicted that the malaria positive case is high among male with 88 positive cases out of 140 male study participants and less in the females with 87 malaria positive cases from 121 female study participants. Also, the sex distribution of malaria parasite by rapid diagnostic test (RDTs) showed that out of 261 study participant tested, 159 were positive for malaria parasite. Male and female were found to be 81 and 78 respectively. This indicated that male participants were highly infected with malaria parasite than females.

Table 2: Sex Distribution of Malaria Parasite by Microscopy and RDTs

		Microscopy		RDTs	
Sex	**No of Samples**	**Positive Samples**	**Negative Samples**	**Positive Samples**	**Negative Samples**
Male	138(52.67%)	88(62.86%)	50(37.16%)	81(57.86%)	57(42.14%)
Female	124(47.33%)	87(69.05%)	37(30.95%)	78(61.90%)	46(38.10%)

There is no relationship between sex of the study participant and being positive of malaria parasite by microscopy or by rapid diagnostic test (RDT). The study revealed the rate of microscopy is not connected with gender because 88(62.86%) of the positive cases detected by microscopy are males with 87(69.05%) females detected by microscopy. Eighty-one (57.86%) of the study participants detected by RDT was males and 78(61.90%) detected by RDT were females. This also showed that the rate of RDT positivity in not connected with gender. This is similar to the findings of Garba *et al.,* in 2006 in Gusau, Nigeria who also found out that the positivity of microscopy and RDT is not connected with gender of the individual [9].

The specificity and sensitivity of 100% with a positive and negative predictive value of 100% were observed. The sensitivity in this study is higher than those reported by Mahende *et al.,* 2016 were they reported a sensitivity of 91.1% in Tanzania [10]. However, this study revealed specificity and positive predictive values similar with those reported by Mahende *et al.,* 2016 in Tanzania [10]. In Tanzania, negative predictive value of 98.8% was reported by Mahende *et al.,* 2016 and this value is lower than 100% obtained in this study on microscopy. Ilesanmi *et al.,* 2017 in Ibadan Nigeria observed 99.6% specificity which higher than the specificity of 93.81% observed with SD Bioline by RDT in this study; but it is lower than the observation made in the study of Awortu *et al.,* in 2007 in Port Harcout Nigeria [12] and Mahende *et al.,* in 2016 in Tanzania who reported the RDT specificity of 100% and 97.8% respectively. Sensitivity of 93.29% of RDT observed in this study is greater than 88.6% sensitivity reported by [10] in Tanzania. Garba *et al.,* 2006 reported very low sensitivity in a study conducted in Gusau Nigeria [9] they reported that RDT had a sensitivity of 9.09% in children under the age of five [5] in Gusau Nigeria. They also reported a positive and negative predictive value of 50.00% and 53.70% respectively. These values are lower than 96.23% and 89.22% obtained as positive and negative predictive values of RDT in our study. In a study conducted by Mahende *et al.,* in 2006 in Tanzania also reported a lower positive predictive value of 84.3 and higher negative predictive values 98.5 compared with our studies [10].

Table 3 showed the comparison of the RDTs results with the result of microscopy using the 2 x 2 contingency table. For microscopy, 170 samples were tested positive while 91 were negative for malaria parasite. While for RDTs 159 samples were tested positive and 102 were tested negative. Among the 170 positive cases diagnosed by microscopy RDT failed to detect 11 cases tested positive by microscopy yielding a false

negative result. Also among the 91 negative cases detected by expert microscopy, 6 cases were RDT positive yielding 6 false positive results. This study confirmed that microscopy remains the reference standard and a better diagnostic tool for malaria diagnosis in the laboratory than the RDTs. High positivity value was obtained in microscopy than RDT. Microscopy has a positivity value of 170 while RDT has a positivity value of 159.

Table 3: Comparison of Microscopy with RDT

	Positive	Negative	True Positive	False positive	True Negative	False negative
Microscopy	170	92	170	0	91	0
RDT	159	103	153	6	91	11

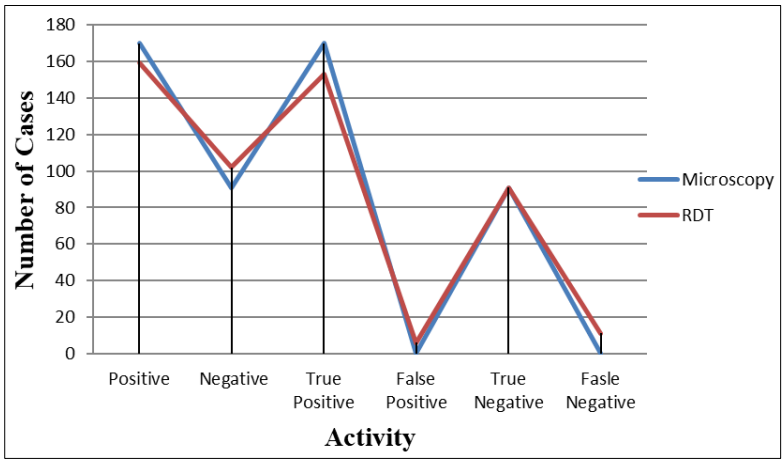

Fig 1: Comparison of Effectiveness between Microscopy and RDT

The test of performance of both microscopy and RDT for the diagnosis of malaria is represented in table 4. The sensitivity, specificity, positive and negative predictive values of microscopy and RDT were calculated and the result indicated that microscopy has sensitivity, specificity, positive and negative predictive values of 100%. However, RDT shows sensitivity of 93.29%, specificity of 93.81%, with positive predictive values of 96.23% and a negative predictive value of 89.22%.

Table 4: Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of Microscopy and RDT

	Sensitivity (%)	Specificity (%)	PPV (%)	NPP (%)
Microscopy	100	100	100	100
RDT	93.29	93.81	96.23	89.22

The sensitivity of 100% in in microscopy is significantly higher than the sensitivity of 93.29% of RDT obtained in this study. The reduced sensitivity of is higher than the 78.4% sensitivity reported by Sheyin and Bigwan in 2013 in Zaria Nigeria [13]. In another research conducted in Zamfara State which is also in northern Nigeria, obtained a very low sensitivity of 40.3% and a specificity of 89.6% in children < 5 years old [14]. Moreover, Brown and Azike, 2014 also reported poor sensitivity of 37.7% and 89.0% Specificity in a study conducted in Port-Harcourt which is located in the southern part of Nigeria [15].

These low sensitivity and specificity of rapid diagnosis techniques could result from two extreme environmental factors which may be as a result of high temperature and humidity in the study areas which eventually can affect the performance of RDTs [16, 17] and the environmental conditions in Zamfara and Port-Harcourt represent these two extremes. Thus in Nigeria there different extreme environmental conditions that are found in different geographical location and can simply interfere with the performance effectivity of the RDTs.

The result in table 5 depicted the prevalence of mixed species of Malaria infection detected by both Microscopy and RDT. Microscopy revealed that 38 sample out of 170 positive samples harbored mixed species of malaria parasite. However, 29 samples out of 159 positive samples detected by RDTs were also found to harbored mixed species of malaria parasite. RDTs variably detect the four *Plasmodium* species that infect humans; depending on the antigens on which they are based. Some RDTs detect *P. falciparum* only, while others detect *P. falciparum* and the other malaria parasites on two separate bands.

Table 5: Mixed Species of Malaria Infection Detected by Microscopy and RDT

	No of Positive Samples	Single Species	Mixed Species
Microscopy	170	132	38
RDT	159	130	29
Total	329	262	67

To date, no commercial RDT has been reported to differentiate reliably between *P. vivax*, *P. Ovale* and *P. malariae*, although research to develop such a test is continuing [5]. This study observed a prevalence of mixed species of Malaria infection detected by both Microscopy and RDT. The species were identified on the basis of their cell morphology as described by Lee *et al.*, 2006 [5]. Microscopy revealed that 38 sample out of 170 positive samples harbored *P. vivax* in addition to *P. falciparum*. However 29 samples out of 159 positive RDTs were found to harbored mixed species.

Conclusion

This study established that microscopy is more efficient and

reliable in the diagnosis of malaria parasites than the rapid diagnostic techniques. Microscopy has a higher sensitivity than rapid diagnostic tests (RDTs). However, RDTs can also serve as useful tools in early diagnosis of malaria in infected persons in other to avert the accompanied burdens associated with late diagnosis.

Recommendation

RDTs would probably be suitable as a potential alternative to field microscopy or for use in the clinics in endemic areas where microscopy is not available or where experts may be lacking. Periodic research should be done on the impact and cost effectiveness of RDTs in providing reliable diagnosis at point of care after implementation and deployment of RDTs in health facilities. However, RDTs should be considered as epidemiological tool for rapid screening of malaria in both endemic and non-endemic settings in order to avoid over or under treatment.

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