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Assessment of Rapid Diagnostic Test and Microscopy in the Detection of *Plasmodium falciparum* Malaria Infection among Young Adults in South-western Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author SSE designed the study, wrote the protocol and the first draft of the manuscript. Authors EI and ENA managed the analyses of the study. Authors OKA and GTF managed the literature searches. Author AOA performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Malaria is a major public health problem in Sub-Sahara Africa with over 95% of occurrence in Nigeria due to *Plasmodium falciparum*. This study assessed the prevalence of *P. falciparum* malaria infection among young adults of Babcock University, Ilishan- Remo, Ogun State, Nigeria using rapid diagnostic test and microscopic methods. A total of 5 ml venous blood was collected from each of

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the 200 consenting randomly recruited young adults (100 males and 100 females) without a history of anti-malaria drugs or herbal remedies in the preceding two (2) weeks. *P. falciparum* in participants' blood was detected using SD Bioline Malaria *P. falciparum* Histidin-Rich Protein II (HRP-II) Antigen Rapid Diagnostic Test Kit supplied by Standard Diagnostics, INC and also by conventional microscopic examination of Giemsa stained thin and thick blood films. The outcome of this study shows that the prevalence of *P. falciparum* malaria infection among young adults of Babcock University as detected by RDT and microscopic method is 0.5% and 86.0%, respectively. The microscopic method appears to be a more reliable diagnostic tool for malaria infection than the rapid diagnostic method with a sensitivity of 86.19%. *P. falciparum* malaria infection was significantly higher ($P<0.05$) among the female participants (47.5%) than their male counterparts (38.5%). And it was also found to be highest among participants within 16-20 years age range (45.5%). 87.8% of the participants who tested positive had a malaria parasite density (MPD) of one plus (+), while the rest (12.2%) had a malaria parasite density of two pluses (++). The findings in this study show that *P. falciparum* malaria infection is common among young adults of Babcock University, Ilishan-Remo, Ogun State, Nigeria, therefore urgent and appropriate public health intervention is required.

Keywords: Malaria; Plasmodium falciparum; rapid diagnostic test; microscopy; risk factors.

1. INTRODUCTION

Malaria is a life-threatening protozoan parasitic disease transmitted through the bite of an infected female anopheles mosquito during a blood meal [1,2]. It is endemic in over 100 countries of the world [3]. In 2015, 15 countries and regions had ongoing malaria transmission, and about 3.2 billion people; almost half of the world populations are at risk of malaria with 300 to 500 million clinical cases occurring annually [4]. In Nigeria, malaria is responsible for over 90% of reported cases of tropical disease, 30% of childhood mortality, 11% of maternal mortality, and more than 50% of outpatient visits [5-7].

There are 5 identified species of malaria parasite that affects human, these include: *Plasmodium falciparium, P. vivax, P. ovale, P. malariae* and *P. knowlesi* [1]. In Nigeria, over 95% of malaria infection is due to *P. falciparum* with *P. ovale* and *P. malariae* playing a minor role, while *P. vivax* is not found among indigenous Nigerians [7].

Early diagnosis of malaria is the cornerstone of malaria treatment and control. However, in a resource scare setting, it has become routine to use a history of subjective fever as the indication to treat for malaria and mismanagement of non-malaria fever, which wastes resources and erodes confidence exist [8]. Nevertheless, there are four principal methods for diagnosis of malaria: clinical, microscopy, antigen test and molecular methods. Laboratory confirmation of malaria infection involves identifying malaria parasites or antigens/products in patient blood and it requires the availability of a rapid, sensitive and specific, test at an affordable cost. World Health Organization recommends prompt parasite-based diagnosis by microscopy or malaria rapid diagnostic test in all patients suspected of malaria before anti-malaria treatment is administered [2,9].

Examination of correctly Giemsa stained thick/thin blood films by microscopy is a basic technique, which remains the gold standard for the diagnosis of malaria. The method requires professional expertise and the availability of a good quality microscope. Importantly, this conventional method allows the identification of different malaria parasite species, various parasite stages, including gametocyte, and the quantification of parasite density to monitor response to treatment [1,10].

On the other hand, the rapid diagnostic tests (RDTs) can also be used for the diagnosis of malaria infection. The test is based on the detection of specific malaria antigens (e.g Histidine rich protein 2, Aldolase and parasite lactate dehydrogenate) derive from malaria parasites in lysed blood using immunochromatographic methods. The result is usually a colored test line, obtained in 5-20 minutes [11]. RDTs are simpler to perform and to interpret, do not require electricity, special equipment or training as in microscopy. However, they are more expensive, not quantitative, do not give detailed information about the parasite, kits that detect, both *P. falciparum* and non-falciparium species cannot differences between *P. vivax* and *P. ovale* malaria, nor can they distinguish pure *P. faciparum* infections from mixed infections that include *P. falciparum* [12].

Accurate diagnosis of malaria cases using microscopy especially at the primary health care level could pose to be a very difficult task, due to lack of good quality microscope, professional expertise in handling microscopy and constant power supply. Since rapid diagnostic test produces a result within a short period of time there is a high preference to the rapid diagnostic kit without recourse to sensitivity, specificity, accuracy and reliability of the result obtained [11,13,14].

Malaria infection caused by *P. falciparum* is common among the young adults of Babcock University, but no documented epidemiological survey has been carried out to determine the prevalence rate. Scarcity of data in this regard poses a serious problem to control and prevention of malaria infection among the student's population. The present study is therefore designed to determine the prevalence of *P. falciparum* infection among young adults of Babcock University, Ogun state, Nigeria using the conventional microscopic and rapid diagnostic method, with emphasis to determine which of the methods is the most effective in the detection of *P. falciparum* infection among the study population.

2. MATERIALS AND METHODS

2.1 Study Design

This is a cross-sectional descriptive study.

2.2 Study Area

This cross-sectional institution-based study was carried among young adults of Babcock University, Ilishan-Remo, Ogun State. Babcock University is a first Class Seventh-day Adventist Institution of higher learning, with a student population of about 6000, located in the south-western region of Nigeria, coordinates: 6.8862ºN, 3.7055ºE.

2.3 Duration of Study

This study was carried out between the months of March and June, 2018.

2.4 Study Population

Young adults of Babcock University were the target population. Currently, the University has a total population of about six thousand, 100-600 level combined They consist of young male and female adults within the age range of 16-35 years from the different ethnic, religious and cultural background; studying different courses in various Departments. The University hosts nine female halls (Nyberg, White, Queen Esther, Felicia Adebisi, Havillah, Gold, Crystal, Ogden, Ameyo Adadevoh and Platinum) and seven male halls (Neal Wilson, Welch, Samuel, Bethel, Nelson Mandela, Gideon Troupers and Winslow). Study participants were randomly selected from the various Halls of Residence.

2.5 Sample size Calculation

The sample size (N) was estimated using the formula described by Charan and Biswas [15]:

$$N = Z^2PQ/d^2$$

where;

N = required sample size,
Z = Standard normal variate at 5% ($p<0.05$) error or 95% confidence interval is 1.96
P = Proportion of the population with malaria parasite infection from previous study,
Q = Proportion of the population without malaria parasite infection (1 – P) and
d = Absolute error margin is 0.05.

For the calculation, a 95% confidence interval, a P value of 0.1407, *i.e,* a prevalence rate of 17.0% from the previous study by Anumudu et al. [16], and margin of error (d) set at 0.05 was used to determine the minimum sample size required. To minimize errors arising from the likelihood of non-compliance, 10% of the sample size was added giving a final sample size of 200.

2.6 Sample Size

A total of 200 blood specimens was collected randomly from consenting students (100 males and 100 females) of Babcock University, Ilishan-Remo, Ogun State.

2.7 Eligibility of Subjects

2.7.1 Inclusion criteria

Consenting male and female young adults of Babcock University without history of anti-malaria

drugs or herbal remedies in the preceding two (2) weeks were randomly recruited for the study.

2.7.2 Exclusion criteria

Young adults with history of anti-malaria drugs or herbal remedies in the preceding two (2) weeks, as well as postgraduate Students of the University were excluded from the study.

2.8 Data Collection

Prior to specimen collection, demographic and clinical information was obtained from participants through the administration of prepared questionnaires and personal interviews. Each questionnaire has a unique participant identification number (PIDN). The first part of the questionnaires contained the biodata of the patients e.g. name, sex, age, study level and marital status. The second part second part included the history of malaria infection (Fever, Chills, Rigors, Body aches, vomiting etc), risk factors (if any), personal hygiene and health care-seeking behaviour. The study population was stratified by sex, age, study level, religion, tribe and marital status. Response to a structured questionnaire administered was used to collect data on epidemiology and demographic trends of malaria infection. For the purpose of privacy, all information obtained from the participants was treated confidentially.

2.9 Specimen Collection

Blood specimen was collected from each participant via venous puncture.

2.10 Laboratory Analyses

Detection of malaria parasite/antigen in the blood sample was carried out using Rapid Diagnosis Test (RDT) and conventional microscopic method.

2.10.1 Detection of malaria parasite using rapid diagnostic method

P. falciparum in Participant's blood (if present) was detected using a one-step Rapid Diagnostic Test (RDT) Device, SD Bioline Malaria *P. falciparum* Histidin-Rich Protein II (HRP-II) Antigen Rapid Diagnostic Test Kit (Fig. 1) supplied by Standard Diagnostics, INC according to the manufacturer instruction.

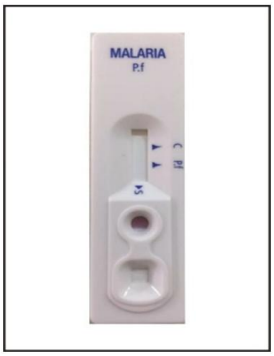

Fig. 1. Picture showing SD bioline malaria *P. falciparum* antigen test cassette

2.10.2 Detection of malaria parasite using the microscopic method

In addition to the Rapid diagnostic method mentioned above, *P. falciparum* in Participant's blood (if present) was also detected in Giemsa stained thin and thick blood films examined using the conventional microscopic method as described by Cheesbrough [17].

2.11 Estimation of the Level of Parasitaemia

To determine the severity of *P. falciparum* infection in each participant, parasite density in thick blood film was estimated using the plus system after the parasite specie has been verified and the morphological stages (gametocyte, trophozoite and schizont) present identified as described by Chiodini et al. [18].

The following plus sign scheme was used to report parasite density:

+ = 1-10 parasites per 100 high power fields (h.p.f)
++ = 11-100 parasites per 100 high power fields (h.p.f)
+++ = 1-10 parasites per single high power field (h.p.f)
++++ = More than 10 parasites per single high power field (h.p.f)

2.12 Data Analysis

Raw data were entered into Microsoft Excel. Statistical analysis was carried out using SPSS Statistics software package (version 18.0). One-way analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test was used to

test for significant differences in the prevalence of *P. falciparum* infection among the students using Rapid Diagnostic and Microscopic method. P values<0.05 was considered significant. The confidence intervals for sensitivity and specificity was computed using the Wilson's score method. A P value ≤ 0.05 was considered statistically significant. Statistical analysis outputs were presented using Tables and charts.

3. RESULTS

The present study investigated the prevalence of *P. falciparum* malaria infection among young adults in south-western Nigeria using Microscopic and Rapid Diagnostic Methods. A total number of 200 young adults (100 males and 100 females) were screened using a rapid diagnostic test (RDT) kits and microscopy. The social demographic characteristics of the participants including gender, age range, marital status, religion, tribe, blood genotype and Hall of residence is presented in Table 1.

The frequency of occurrence of *P. falciparum* malaria infection among the study participants using Rapid Diagnostic and microscopic method is presented in Table 2 and 3, respectively. Out of the 200 participants screened, only 1 person (0.5%) was positive for *P. falciparum* infection by RDT method, while 172 (86.0%) were positive by Microscopic method.

Based on gender distribution, percentage malaria positivity among female participants were 0.5% and 47.5% by RDT and microscopic methods, respectively; while it was 0% and 38.5%, respectively by the same methods for male participants. *P. falciparum* malarial infection appears to be significantly higher (P<0.05) among the female participants than their male counterparts.

On the basis of age distribution, the data shows that only 1 (0.5%) out of 93 participants within the age group: 21-25 years was positive for *P. falciparum* infection, while others were negative by the rapid diagnostic method. On the other hand, the highest prevalence rate (45.5%) occurred among participants within 16-20 years age range, 91 out of the 102 participants examined were found to be positive by microscopic method. The percentage positivity for *P. falciparum* infection among this age group was found to be significantly higher (P<0.05) than other age groups.

With regards to their marital status, all the 200 study participants examined were single (100%), none was married (0%). Only 1 (0.5%) was found positive by RDT method, while 172 (86.0%) were found to be positive by the microscopic method. Based on their blood genotype, the highest occurrence of *P. falciparum* infection (59.0%) was found among those with blood genotype “AA”, with *p-value*<0.05, considered statistically significant; while the lowest was found among those with genotype “AC” (0.5%) by microscopy. On the other hand, only 1 person with “AA” genotype was positive for *P. falciparum* infection by RDT method.

Furthermore, with regard to their hall of residence, Havillah Hall, Ameyo Adadevoh Hall, Welch Hall and Bethel Hall recorded significant (P<0.05) occurrence of *P. falciparum* infection among the occupants than others using the microscopic method. Meanwhile, 0.5% prevalent rate was found in Ameyo Adadevoh Hall using RDT method.

The relationship between the occurrence of *P. falciparum* and indications for malaria infection is presented in Fig. 2. Majority of the participants who tested positive to *P. falciparum* by microscopy indicated varied signs and symptoms that were consistent with malarial infection. Ninety-seven (97; 56.4%) complained of loss of appetite, which was found to be significantly higher (P<0.05) than other signs and symptoms: back and joints pain (14.5%), headache (8.7%), fever (8.1%), diarrhea (4.7%), vomiting (2.9%), as well as sweating and chills (1.7%).

Table 4 shows the distribution of symptomatic and asymptomatic *P. falciparum* infection among the study participants. While 81 (47.1%) out of the 172 participants who tested positive to *P. falciparum* by microscopy were symptomatic, 91 (52.9%) were symptomless. On the basis of gender, the prevalence of symptomatic malaria infection was significantly higher (P<0.05) among female Subjects (61.7%) than in males (38.3%), whereas there was no significant difference (P>0.05) in the prevalence of asymptomatic malaria infection between the females (49.5%) and males (50.5%).

Based on the Age range, the highest prevalence for symptomatic infection was found among 16-20Years (51.9%) and the lowest among 26-30 Years (1.2%). Similarly, the same pattern was observed for asymptomatic infection: 53.8% and 1.1%, respectively. The differences were statistically significant (P<0.05).

Based on blood genotype, symptomatic malaria infection was highest (P<0.05) among the "AA" genotype (66.7%), but lowest among "AC" genotype (1.2%). Similarly, asymptomatic malaria infection was also more prevalent (P<0.05) among "AA" genotype (70.3%), followed by "AS" genotype (24.2%). Meanwhile asymptomatic malaria infection was completely absent among "AC" genotype (0%).

Analysis of distribution of malarial infection based on the Hall of residence, shows that symptomatic and asymptomatic infection were both highest in Havilah hall, 25.9%, 22.0%, respectively; where-as Bethel (1.2%) and Winslow (1.1%) recorded the lowest symptomatic and asymptomatic malaria infection, respectively.

The severity of *P. falciparum* infection by conventional microscopic method is presented in Table 5. Regardless of their demographic factors, most of the participants (87.8%) had a malaria parasite density (MPD) of one plus (+), while the rest (12.2%) had a malaria parasite density of two pluses (++). None (0%) had MPD of three (+++) or four (++++) pluses. It was also noted that 11.1% of the female participants had a MPD of two pluses (++), which was found to be statistically significant (P<0.05) than the 1.2% observed among the males. The highest occurrence of MPD of two pluses (++) was found among participants of 21-25 years (7.6%), followed by 16-20 years (4.7%), while none (0%) was recorded for 26-30 years.

Table 1. Demographic characteristics of the study participants

Characteristics	Category	Number (%)
Gender	Male	100 (50.0)
	Female	100 (50.0)
	Total	200 (100)
Age range	16-20 Yrs	102 (51.0)
	21-25 Yrs	93 (46.5)
	26-30 Yrs	5 (2.5)
	Total	200 (100)
Marital status	Single	200 (100)
	Married	0 (0)
	Total	200 (100)
Religion	Christianity	192 (96.0)
	Islam	3 (1.5)
	Traditional	0 (0)
	Other	5 (2.5)
	Total	200 (100)
Tribe	Yoruba	111 (55.5)
	Igbo	43 (21.5)
	Hausa	8 (4.0)
	Others	38 (19.5)
	Total	200 (100)
Blood genotype	AA	137 (68.5)
	AS	43 (21.5)
	SS	0 (0)
	AC	1 (0.5)
	Not known	19 (9.5)
	Total	200 (100)
Hall of residence	Ameyo-Adadevoh Hall	27 (13.5)
	Havilah Hall	42 (21.0)
	White Hall	31 (15.5)
	Gideon Hall	21 (10.5)
	Welch Hall	26 (13.0)
	Neal Wilson Hall	21 (10.5)
	Samuel Akande Hall	23 (11.5)
	Bethel Hall	3 (1.5)
	Winslow Hall	6 (3.0)
	Total	200 (100)

Table 2. The frequency of occurrence of *P. falciparum* malaria infection using rapid diagnostic test method

Characteristics	Number examined (%)	Number positive (%)	Number negative (%)	*P-value*
Gender				
Male	100 (50.0)	0 (0)	100 (50.0)	0.500
Female	100 (50.0)	1 (0.5)	99 (49.5)	0.500
Total	200 (100)	1 (0.5)	199 (99.5)	
Age range				
16-20Yrs	102 (51.0)	0 (0)	102 (51.0)	0.490
21-25Yrs	93 (46.5)	1 (0.5)	92 (46.0)	0.465
26-30Yrs	5 (2.5)	0 (0)	5 (2.5)	0.975
Total	200 (100)	1 (0.5)	199 (99.5)	
Blood genotype				
AA	137 (68.5)	1 (0.5)	136 (68.0)	0.685
AS	43 (21.5)	0 (0)	43 (21.5)	0.785
SS	0 (0)	0 (0)	0 (0)	0
AC	1 (0.5)	0 (0)	1 (0.5)	0.995
Not known	19 (9.5)	0 (0)	19 (9.5)	0.905
Total	200 (100)	1(0.5)	199 (99.5)	
Hall of residence				
Female Halls				
Ameyo Adadevoh Hall	27 (13.5)	1 (0.5)	26 (13.0)	0.135
Havilah Hall	42 (21.0)	0 (0)	42 (21.0)	0.790
White Hall	31 (15.5)	0 (0)	31 (15.5)	0.845
Male Halls				
Gideon hall	21 (10.5)	0 (0)	21 (10.5)	0.895
Welch	26 (13.0)	0 (0)	26 (13.0)	0.870
Neal Wilson	21 (10.5)	0 (0)	21 (10.5)	0.895
Samuel Akande	23 (11.5)	0 (0)	23 (11.5)	0.885
Bethel	3 (1.5)	0 (0)	3 (1.5)	0.985
Winslow	6 (3.0)	0 (0)	6 (3.0)	0.970
Total	200 (100)	1 (0.5)	199 (99.5)	

P value >0.05 is considered statistically not significant

Similarly, the highest occurrence of MPD of two pluses (++) was recorded among participants with "AA" genotype (7.6%), followed by "AS" and "AC" genotypes, 3.5% and 0.6%, respectively. With regard to their Hall of residence, participants with MPD of two pluses (++) were significantly (P<0.05) found in Havillah hall (5.2%), followed by Ameyo-Adedevoh hall (2.9%), White hall (2.9%) and Gideon hall (1.2%), while the rest of the hall had MPD of one plus (+) only.

Risk factors associated with the occurrence of *P. falciparum* infection among the participants is presented in Table 6. 55 (32%) participants who tested positive by microscopy had no awareness and knowledge of *P. falciparum* as causative agent of malaria. Also 159 (92.4%) of them had history of malarial infection.

One hundred forty-eight (148, 86%) claimed that they have no mosquito bed nets. 132 (77.2%) indicated that they never slept under mosquito bed net. 41 (23.8%) indicated they do not use insecticides at all. Regarding frequency of spraying, 65 (37.8%) said they sprayed their rooms with insecticides less often. Eight (8, 4.7%) indicated the absence of window nets, whereas 25 (14.5%) indicated that the window nets are in a bad condition. 66 (38.4%) indicated the presence of stagnant water around their Hall of residence. 3 (1.7%) indicated that the vegetation around the hostel was never cleared and also 54 (31.4%) indicated that the vegetation is cleared less often.

Furthermore, 85 (49.4%) indicated that they stay late outside at night very often. 28 (16.3%) mentioned that they never wear protective clothing when outside at night. 38 (22.1%) indicated they never wear protective clothing to bed, whereas 39 (22.7%) indicated that they do, but less often. Finally regarding attitude towards laboratory test and medical checkup, 125 (72.7%) of the participants positive by microscopic method indicated they go for laboratory test and medical checkup less often. Only 8 (4.7%) of them indicated very often.

Table 7 shows the sensitivity, specificity and predictive values of the two diagnostic methods used in this study. Going by the Rapid Diagnostic method, only one (1) participant was truly positive (TP), nobody (0) was false positive (FP), nineteen (19) were truly negative (TN), one hundred and eighty (180) were false negative. Meanwhile, the method had 0.6% Sensitivity, 100% Specificity, 100% positive predictive value (PPV) and 9.55% negative predictive value (NPV).

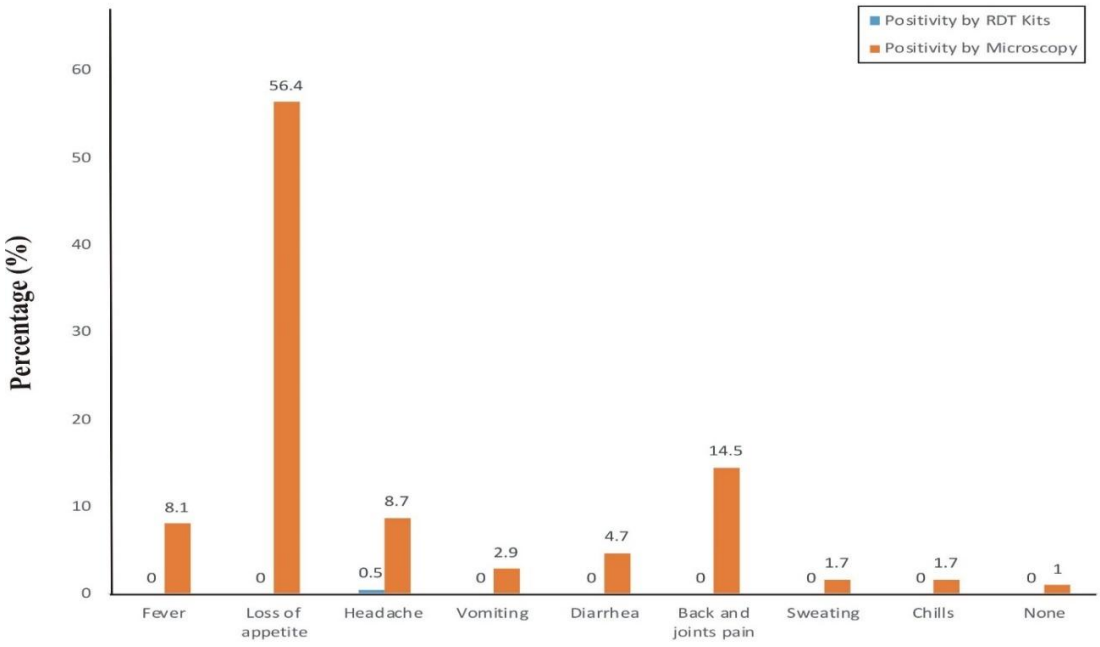

Fig. 2. Relationship between the detection of *P. falciparum* and indications for malaria infection

Table 3. Frequency of occurrence of *P. falciparum malaria* infection using microscopic method

Characteristics	Number examined (%)	Number positive (%)	Number negative (%)	*P-value*
Gender				
Male	100 (50.0)	77 (38.5)	23 (11.5)	0.100
Female	100 (50.0)	95 (47.5)	5 (2.5)	0.005*
Total	200 (100)	172 (86.0)	28 (14.0)	
Age Range				
16-20Yrs	102 (51.0)	91 (45.5)	11 (5.5)	0.028*
21-25Yrs	93 (46.5)	79 (39.5)	14 (7)	0.121
26-30Yrs	5 (2.5)	2 (1.0)	3 (1.5)	0.250
Total	200 (100)	172 (86.0)	28 (14.0)	
Blood genotype				
AA	137 (68.5)	118 (59.0)	19 (9.5)	0.032*
AS	43 (21.5)	40 (20.0)	3 (1.5)	0.101
SS	0 (0)	0 (0)	0 (0)	0
AC	1 (0.5)	1 (0.5)	0 (0)	0.860
Not known	19 (9.5)	13 (6.5)	6 (3.0)	0.548
Total	200 (100)	172 (86.0)	28 (14.0)	
Hall of residence				
Female Halls				
Ameyo Adadevoh	27 (13.5)	26 (13.0)	1 (0.5)	0.007*
Havillah	42 (21.0)	41 (20.5)	1 (0.5)	0.008*
White	31 (15.5)	28 (14.0)	3 (1.5)	0.333
Male Halls				
Gideon	21 (10.5)	18 (9.0)	3 (1.5)	0.591
Welch	26 (13.0)	19 (9.5)	7 (3.5)	0.049*
Neal Wilson	21 (10.5)	16 (8.0)	5 (2.5)	0.149
Samuel Akande	23 (11.5)	18 (9.0)	5 (2.5)	0.201
Bethel	3 (1.5)	3 (1.5)	0 (0)	0.037*
Winslow	6 (3.0)	3 (1.5)	3 (1.5)	0.634
Total	200 (100)	172 (86.0)	28 (14.0)	

**P value <0.05 is considered statistically significant*

Table 4. The distribution of symptomatic and asymptomatic *P. falciparum* malaria Infection

Demographic characteristics	Number with symptomatic *P. falciparum* infection N (%)	Number with asymptomatic *P. falciparum* infection N (%)	Number without *P. falciparum* infection N (%)	*P-value*
Gender				
Male	31 (38.3)	46 (50.5)	23 (82.1)	0.213
Female	50 (61.7)	45 (49.5)	5 (17.9)	0.001*
Total	81 (100)	91 (100)	28 (100)	
Age range				
16-20Yrs	42 (51.9)	49 (53.8)	11 (39.3)	0.044*
21-25Yrs	38 (46.9)	41 (45.1)	14 (50)	0.041*
26-30Yrs	1 (1.2)	1 (1.1)	3 (10.7)	0.510
Total	81 (100)	91 (100)	28 (100)	
Blood genotype				
AA	54 (66.7)	64 (70.3)	19 (67.9)	0.050*
AS	18 (22.2)	22 (24.2)	3 (10.7)	0.977
SS	0 (0)	0 (0)	0 (0)	0
AC	1 (1.2)	0 (0)	0 (0)	0.478
Not known	8 (9.9)	5 (5.5)	6 (21.4)	0.706
Total	81 (100)	91 (100)	28 (100)	
Hall of residence				
Ameyo-Adadevoh	16 (19.8)	10 (11)	1 (3.6)	0.028*
Havillah	21 (25.9)	20 (22)	1 (3.6)	0.001*
White	13 (16)	15 (16.4)	3 (10.7)	0.025*
Gideon	9 (11.1)	9 (9.8)	3 (10.7)	0.071
Welch	8 (9.8)	11 (12.1)	7 (25)	0.066
Neal Wilson	5 (6.2)	11 (12.1)	5 (17.9)	0.095
Samuel Akande	6 (7.4)	12 (13.2)	5 (17.9)	0.283
Bethel	1 (1.2)	2 (2.2)	0 (0)	0.161
Winslow	2 (2.5)	1 (1.1)	3 (10.7)	0.155
Total	81 (100)	91 (100)	28 (100)	

**P-value <0.05 is considered statistically significant*

For the microscopic method, one hundred and fifty-six (156) participants were truly positive (TP), sixteen (16) were false positive (FP), three (3) were truly negative (TN), twenty-five (25) were false negative. Meanwhile, the method had 86.2% Sensitivity, 15.8% Specificity, 90.7% positive predictive value (PPV) and 10.7% negative predictive value (NPV).

4. DISCUSSION

Malaria is a serious public health concerns in tropical and sub-tropical countries [19], with most cases in Nigeria caused by *P. falciparum* because of its wide spectrum [20-22]. Early diagnosis of malaria is the cornerstone of malaria treatment and control [8]. The present study investigated the prevalence of *P. falciparum* malaria infection among Young adults of Babcock University, Ilishan-Remo, Ogun State, Nigeria using Rapid Diagnostic and Conventional Microscopic Method, with emphasis to determine which of the methods is the most effective in the detection of *P. falciparum* infection among the study population.

A total number of 200 young adults (100 females and 100 males) were screened. Out of the 200 participants screened, only 1 person (0.5%) was positive by the Rapid Diagnostic Test Method, while 172 (86.0%) were positive by the Microscopic Method. The results of the present study agree with those of previous studies which affirm that a higher occurrence rate of *Plasmodium falciparum* malaria infection is detectable by microscopic method, rather than by Rapid Diagnostic Test Method.

For instance, Ruqayyah et al. [22], reported a prevalence rate of 18.6% and 56.5% using the Rapid Diagnostic Test and Microscopic Method, respectively, in North-Western Nigeria. Also, Oyetunde *et al.* [23], reported a prevalence of 36.8% and 66.8% among febrile patients in Ijebu-Ode, Ogun State, South-Western Nigeria using RDT and malaria microscopic method, respectively. The outcome of the work shows that the detection and prevalence of malaria infection by Microscopic Method is high according to WHO endermicity classification.

Furthermore, the prevalence of malaria infection in this present study (86.0%) by microscopic method was found to be higher than those of previous studies. For instance, Kapesa et al. [24] reported a prevalence of 26.6% among Kenyan school children. Among Nigerian populace, Ruqayyah et al. [22], Oyetunde et al. [23] and Kalu et al. [20], reported a prevalence of 56.5%, 66.8% and 80.4%, respectively.

It was also found to be higher than the 64% reported by Ezugbo-Nwobi et al. [25] among young adults of Nnamdi Azikiwe University, Awka, Anambra State in Eastern Nigeria. Still it was higher than the 71% and 80.3% reported by Adepeju [26], as well as Simon-ole and Obimakinde [27] among young adults of Federal University of Technology, Akure, Ondo State in South-Western Nigeria, respectively.

On the other hand, the outcome of this study contradicts that of Garba et al. [28], who reported a lower prevalence of 7.5% among blood donors in Kaduna, North-Central Nigeria using Microscopic Method. Still, malaria percentage positivity observed in this current study was also found to be higher than the 26.6% reported by Kapesa et al. [29] among Kenyan school children.

With respect to the prevalence of malaria infection on the basis of gender, Gilles and Warren [30], claimed that there was no scientific evidence to prove that higher prevalence is influenced by gender as both male and female stand equal chances of been bitten by the insect vector (female anopheles mosquitoes) and getting infected by malaria parasite. Nevertheless, the present data show that the prevalence of *P. falciparum* malaria infection by the rapid diagnostic method was 0% and 0.5% for male and female, respectively, while it was 38.5% and 47.5%, respectively, by microscopic method. The result obtained here shows that the proportion of females infected with malaria infection were significantly higher ($p<0.05$) than males. This agrees with the findings from previous studies like that of Kalu et al. [20] and Ibekwe [31] conducted in South-Eastern Nigeria, where females were found to be more infected than their male counterparts. Also, Olatunji [32], reported that the prevalence of malaria infection was higher in females than in males.

On the other hand, it contradicts reports by Adeyemo et al. [33] and Ruqayyah et al. [22], which both clearly indicated that malaria infection is more prevalent among male subjects than their female counterparts. For instance, the former, reported the prevalence of 55.3% and 44.7% for

Table 5. The severity of *P. falciparum malaria* infection by conventional microscopic method

Characteristics	Number positive (%)	Parasite load				*P-value*
		+	++	+++	++++	
Gender						
Male	77 (44.8)	75 (43.6)	2 (1.2)	0 (0)	0 (0)	0.100
Female	95 (55.2)	76 (44.2)	19(11.1)	0 (0)	0 (0)	0.050*
Total	172 (100)	151(87.8)	21(12.2)	0 (0)	0 (0)	
Age range						
16-20Yrs	91 (52.9)	83 (48.3)	8 (4.7)	0 (0)	0 (0)	0.143
21-25Yrs	79 (45.9)	66 (38.4)	13 (7.6)	0 (0)	0 (0)	0.010*
26-30Yrs	2 (1.2)	2 (1.2)	0 (0)	0 (0)	0 (0)	0.271
Total	172 (100)	151 (87.8)	21(12.2)	0 (0)	0 (0)	
Blood genotype						
AA	118 (68.6)	105 (61.1)	13 (7.6)	0 (0)	0 (0)	0.014*
AS	40 (23.3)	34 (19.8)	6 (3.5)	0 (0)	0 (0)	0.267
SS	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0
AC	1 (0.6)	0 (0)	1 (0.6)	0 (0)	0 (0)	0.061
Not known	13 (7.6)	12 (7.0)	1 (0.6)	0 (0)	0 (0)	0.777
Total	172 (100)	151 (87.8)	21 (12.2)	0 (0)	0 (0)	
Hall of residence						
Ameyo Adadevoh hall	26 (15.1)	21 (12.2)	5 (2.9)	0 (0)	0 (0)	0.032*
Havilah hall	41 (23.8)	32 (18.6)	9 (5.2)	0 (0)	0 (0)	0.004*
White hall	28 (16.3)	23 (13.4)	5 (2.9)	0 (0)	0 (0)	0.035*
Gideon hall	18 (10.5)	16 (9.3)	2 (1.2)	0 (0)	0 (0)	0.048*
Welch	19 (11.0)	19 (11.1)	0 (0)	0 (0)	0 (0)	0.448
Neal Wilson	16 (9.3)	16 (9.3)	0 (0)	0 (0)	0 (0)	0.130
Samuel Akande	18 (10.5)	18 (10.5)	0 (0)	0 (0)	0 (0)	0.145
Bethel	3 (1.7)	3 (1.7)	0 (0)	0 (0)	0 (0)	0.610
Winslow	3 (1.7)	3 (1.7)	0 (0)	0 (0)	0 (0)	0.117
Total	172 (100)	151 (87.8)	21 (12.2)	0 (0)	0 (0)	

*Key: + = 1-10 parasites per 100 high power fields (h.p.f), ++ = 11-100 parasites per 100 high power fields (h.p.f), +++ = 1-10 parasites per single high power field (h.p.f), ++++ = More than 10 parasites per single high power field (h.p.f). *P value <0.05 is considered statistically significant*

male and female subjects respectively, among Students of the University of Benin, Edo State, Nigeria, while the latter, reported 19.4% and 17.9%, respectively, using RDT method and 58.0% and 55.3%, respectively, using microscopic method. It also contradicts the work of Ezugbo-Nwobi et al. [25], who reported no significant differences (P"0.05) between male (64.6%) and female (63.3%) students of Nnamdi Azikwe University, South-Eastern Nigeria.

On the basis of age distribution, the outcome of this study showed that participants within16-25 years age range were more infected with *P. falciparum* malaria infection than other age range which agrees with the reports of previous studies. For instance, Adesina [34], reported a higher prevalence among 17-19 years Students of University of Maiduguri, North-Eastern Nigeria. Meanwhile, Adepeju [26], recorded the highest prevalence among <20 years Subjects and the least among 25-27 years Subjects. In addition, the 45.5% prevalence rate recorded among 16-20years young adults in this current study was lower than the 66.9% reported by Ezugbo-Nwobi et al. [25], among 17-21years students of Nnamdi Azikiwe University, Awka, Anambra State in Eastern Nigeria, but higher than the 41.2% reported by Kapesa et al. [29] among school children aged 5–14 years.

With regard to their blood genotype, the current data indicates that there is significant association between the "AA" blood genotype and occurrence of *P. falciparum* malaria infection. The outcome of this present study agrees with that of Ebong et al. [35], who reported the highest prevalence of malaria infection among individuals with "AA" genotype (82.1%), followed by "AS" genotype (15.4%) and lastly "SS" genotype (2.5%) in his work titled, "Is this evidence of success in malaria prevention and control measures?" It has been posited that malaria parasites tend to thrive more successfully in red blood cells with the normal hemoglobin, hence, the plausible reason for higher occurrences of *P. falciparum* malaria infection among individuals with "AA" blood genotype.

Although there was no record of "SS" blood genotype in this study, carriers of the sickle cell (haemoglobins) have been reported to be less susceptible to *P. falciparum* malaria than non-sicklers. Sickle cell trait is very common in Africa where falciparum infection is hyperendemic, howbeit *P. falciparum* does not multiply properly in sickle red cells containing the abnormal haemoglobin S. The sickle cell trait, which is otherwise undesirable, appears to be conserved in Africa because of the survival advantage it offers in falciparum malaria [35, 36].

Furthermore, majority of the participants who tested positive to *P. falciparum* by microscopy indicated varied signs and symptoms that were consistent with malarial infection. 97 (56.4%) complained of loss of appetite, which was found to be significantly higher (P<0.05) than others: back and joints pain (14.5%), headache (8.7%), fever (8.1%), diarrhea (4.7%), vomiting (2.9%), sweating and chills (1.7%). These results are slightly different from that of Olusegun-joseph [37], who identified headache (51.5%), fever (26%), dizziness (9.5%), Vomiting (6.0%), body pain (5.0%) and loss of appetite (2.0%) as the major signs and symptoms of malaria in a survey among Students of University of Lagos, Nigeria.

From Table 4, this study revealed that there was no significant difference (P>0.05) between the number of males (50.5%) and females (49.5%) with asymptomatic *P. falciparum* malaria infection. The study agrees with the report of previous studies like that of Adepeju [26], who made similar observation: males (59.0%) and females (46.3%). This study also shows that more females (61.7%) had symptomatic *P. falciparum* malaria infection than their male counterparts (38.3%). This observation, however, contradicts the report of previous study by Adepeju [26] who observed no significant difference (P>0.05) between the number of males (94.4%) and females (93.6%) with symptomatic *P. falciparum* malaria infection.

In addition, on the basis of age category, the highest prevalence for symptomatic infection was found among 16-20 years participants(51.9%), which was significantly higher (P<0.05) than the age group with the lowest number of participants: 26-30 Years (1.2%). This observation does not agree with the reports of Adepeju [26], who observed no significant difference (P>0.05) between and within the various age groups examined. Similarly, the same pattern was observed for asymptomatic infection: 53.8% and 1.1% for 16-20Years and 26-30 Years, respectively. The difference was statistically significant (P<0.05). This observation is in discordance with the work of Adepeju [26], who observed no significant difference (P>0.05) in the prevalent of asymptomatic infection between and within the various age groups examined. Overall,

the 45.5% asymptomatic malaria positivity observed in this study was found to be higher than the 6.4% and 38.3% reported by Kapesa et al. [29] among Kenyan school children in low and high transmission settings, respectively.

With regard to the prevalence of malaria infection by Hall of residence, the location of the female hostels in particular and the associated environmental factors such as stagnant water in or around some of them may be responsible for the high prevalence of malaria recorded among some of the occupants. 38.4% of the participants who tested positive for malaria by microscopic method indicated presence of stagnant water in and around their halls of residence. No doubt, presence of stagnant water around dwelling places is breeding sites for mosquitoes and has been associated with high prevalence of malaria infection in the Sub-Sahara Africa [33,34]. It was also observed from the data obtained in this study that 31.4% of participants who tested positive to malaria by microscopy indicated that the bushy vegetation around their halls of residence are cleared less often. Malaria endemicity has been linked to bushy and dirty environment [37].

Associated risk factors identified in this study are similar and comparable with those reported by Kalu et al. [20], Adeyemo et al. [33], Ferreira et al. [38], Olasehinde et al. [39], Degefa et al. [40] and Ochomo et al. [41]. This explains the endermicity of malaria in Africa and this also agrees with the World Health Organization (WHO) early observation that *P. falciparum* causes majority of the malaria infection in Nigeria.

The high prevalence of malaria infection recorded in this current study may further be attributed possibly to the use of substandard insecticides and antimalarial drugs in circulation to which the anopheles mosquitoes and malaria parasite may have developed resistance to respectively, as evident by high malaria parasitemia even among those who claimed to be using insecticides and taking antimalarial drugs as prophylaxis.

Normally, insecticides are used to kill mosquitoes and protect members of the communities from mosquito bites. No (or low numbers of) mosquito bites mean no or less risk of malaria. However, after repeated application of these chemicals, the mosquitoes develop insecticide resistance, which means that they are no longer killed by the insecticides, hence a possible reduction in the efficacy of malaria vector control [41].

Similarly, the presence of substandard antimalarial drugs in circulation, combined with the menace of drug abuse through self-medication, may have contributed to the development of resistance in the parasite itself. The case is even made more worst, when substandard drugs and insecticides are left unchecked in circulation by the appropriate regulatory agencies like: Standard Organization of Nigeria (SON), Consumer Protection Council (CPC) and National Agency for Food and Drug Administration and Control (NAFDAC). The implications of this laxity is: 1) The parasite develops intrinsic ability to survive inside the human host. 2) Can no longer be killed and patients cannot be cured unless new drugs are developed and introduced for treatments. 3) a large number of the malaria parasite and the insect vector (mosquitoes) will survive in the community, and the risk of malaria infections rises and many more people become infected and affected.

Another issue is the non-use or discontinuous use of insecticides by some of the participants to get rid of malaria vector in their halls of residence due to incidence and history of insecticide-induced allergy they have suffered in the past. Some of the participants claim that some insecticides have unpleasant smell with adverse effect on their health. Hence their reason for non-use or discontinuous use of insecticides and therefore a higher risk of been bitten by mosquito and becoming infected with malaria parasite upon exposure.

With regard to the performance of the two methods employed in this study, current data show that the rapid diagnostic method was more specific but less sensitive, while the microscopic method was more sensitive, but less specific than the former. Though the specificity of microscopy in this study was not as high as that of RDT; nevertheless, it has high sensitivity, a possibility for quantification of parasitaemia, and easy handling which is a good advantage and these differ from the findings of Olusola et al. [42], who reported a sensitivity of 62.3%, specificity of 87.4%, positive predictive value of 67.7% and negative predictive value of 84.5% using microscopic method, while a sensitivity of 77.2%, specificity of 72%, positive predictive value of 66.9% and a negative predictive value of 81.1% using rapid diagnostic method.

Table 6. Risk factors associated with the occurrence of *P. falciparum* malaria infection

Risk factors	Responses	No. of participants (%)	No. positive by rapid diagnostic method (%)	No. positive by microscopic method (%)	*P-value*
Awareness/knowledge about *P. falciparum*	Yes	134(67)	0 (0)	117(68)	0.041*
	No	66(33)	1(100)	55(32)	
History of malaria infection	Yes	185(92.5)	1(100)	159(92.4)	0.022*
	No	15(7.5)	0 (0)	13(7.6)	
Availability of mosquito bed net	Yes	28(14)	1(100)	24(14)	0.088
	No	172(86)	0 (0)	148(86)	
Frequency of sleeping under the mosquito net	Never	157(78.5)	0 (0)	132(77.2)	0.018*
	Often	11(5.5)	1(100)	11(6.4)	
	Less often	28(15)	0 (0)	25(14.6)	
	Very often	4(2)	0 (0)	3(1.8)	
Use of insecticide	Yes	149(74.5)	1(100)	131(76.2)	0.029*
	No	51(25.5)	0 (0)	41(23.8)	
Frequency of spraying the room	Never	41(20.5)	0(0)	34(19.8)	0.599
	Often	65(32.5)	0 (0)	54(31.4)	
	Less often	75(37.5)	1(100)	65(37.8)	
	Very often	19(9.5)	0 (0)	19(11)	
Availability of window net	Yes	191(95.5)	1(100)	164(95.3)	0.081*
	No	9(4.5)	0 (0)	8(4.7)	
Present condition of the window net	Very good	157(78)	0 (0)	133(77.3)	0.018*
	Good	11(5.5)	1(100)	11(6.4)	
	Bad	28(14)	0 (0)	25(14.5)	
	Very bad	4(2)	0 (0)	3(1.7)	
Stagnant water around hostel	Yes	74(37)	1(100)	66(38.4)	0.497
	No	126(63)	0 (0)	106(61.6)	
Frequency of clearing vegetation	Never	3(1.5)	0 (0)	3(1.7)	0.805
	Often	108(54)	0 (0)	90(52.3)	
	Less often	60(30)	1(100)	54(31.4)	
	Very often	29(14.5)	0 (0)	25(14.5)	
Frequency of staying late outside at night	Never	16(8)	1(100)	14(8.1)	0.978
	Often	23(11.5)	0 (0)	21(12.2)	
	Less often	63(31.5)	0 (0)	52(30.2)	
	Very often	98(48)	0 (0)	85(49.4)	

Risk factors	Responses	No. of participants (%)	No. positive by rapid diagnostic method (%)	No. positive by microscopic method (%)	*P-value*
Frequency of wearing protective clothing when outside at night	Never	33(16.5)	0 (0)	28(16.3)	0.625
	Often	83(41.5)	1(100)	68(39.5)	
	Less often	67(33.5)	0 (0)	62(36)	
	Very often	17(8.5)	0 (0)	14(8.1)	
Frequency of wearing protective clothing to bed	Never	41(20.5)	0 (0)	38(22.1)	0.748
	Less often	43(21.5)	0 (0)	39(22.7)	
	Often	104(52)	1(100)	85(49.4)	
	Very often	12(6)	0 (0)	10(5.8)	
Frequency of laboratory test/ medical checkup	Less often	148(74)	0 (0)	125(72.7)	0.536
	Often	43(21.5)	1(100)	39(22.7)	
	Very often	9(4.5)	0 (0)	8(4.7)	

**P value <0.05 is considered statistically significant*

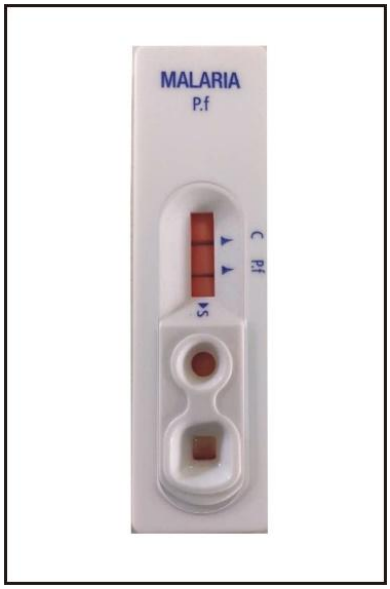

Fig. 3. Picture showing a SD Bioline Malaria Test Cassette positive for *P. falciparum* Histidin-Rich Protein II (HRP-II) Antigen

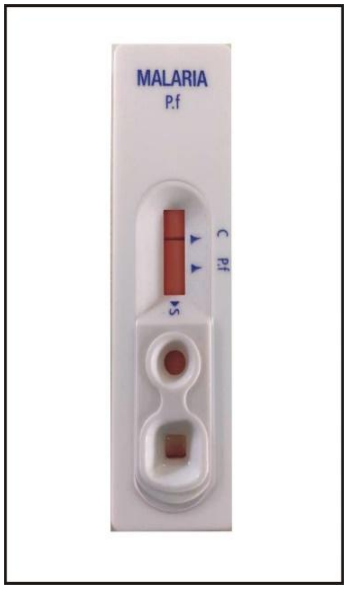

Fig. 4. Picture showing a SD Bioline Malaria Test Cassette negative for *P. falciparum* Histidin-Rich Protein II (HRP-II) Antigen.

Furthermore, Tseroni et al. [43] assess the field application of the RDT for the *P. vivax* diagnosis in comparison to light microscopy and polymerase chain reaction. According to Tseroni et al. [43], the majority of RDT tests show high level of Plasmodium detection at parasitemia of at least 2000 parasites/ul. Therefore, it can be deduced that the low prevalence rate of *P. falciparum* infection reported in this study using RDT may be due to the detection of low parasite densities (+ and ++ parasite loads) in the study participants, which were below the detection limit for our kit.

On this note, there has been a continuous strive to develop more portable, stable, sensitive and low cost detection system for malaria to meet the demand of effective screening actions in developing countries where the disease is most endemic. Singh et al. [44] for instance, described the dye Coupled Aptamer-Captured Enzyme Catalyzed Reaction for the detection of Pan Malaria and *P. falciparum* Species in Laboratory Settings and Instrument-Free Paper-Based Platform. This developed method and technique offered detection of the biomarkers within a clinically relevant dynamic range, with the limit of detection values in the picomolar level. An aptamer-based field effect transistor biosensor, developed by using an extended gate field effect transistor with inter-digitated gold microelectrodes for the detection of the malaria biomarker *P. falciparum* glutamate dehydrogenase in serum samples was also reported by Singh et al. [45]. This biosensor exhibited a sensitive response in broad dynamic range of 100 fM -10 nM with limits of detection of 16.7 pM and 48.6 pM in spiked buffer and serum samples, respectively. Overall, their results validated the application potential of the developed aptaFET for diagnosis of both symptomatic and asymptomatic malaria.

Overall, the outcome of this study agrees with the work of Mac et al. [2] and Alade et al. [9] who both affirmed that microscopy is more sensitive and reliable than RDT for all screened cases of malaria infection. The high false negative result associated with RDT as previously posited may be due to the deletion or mutation of histidine rich protein gene in the parasite, hence the reason for the low sensitivity. Besides, environmental conditions in the manufacturing process of RDT kits, including product storage temperature and shelf life may explain some of the pitfalls. According to Mac et al. [2], the limitations of RDT include its limited ability to detect 100 parasites/µl of all Plasmodium species and its inability to carry out semi-quantitative measurements to monitor the results of drug treatment. New technologies should therefore be improved on in order to develop new devices that are comparable to Microscopy which is the gold standard.

Table 7. Sensitivity, specificity and predictive values of rapid diagnostic method and microscopic method

Diagnostic method	TP (No)	FP (No)	TN (No)	FN (No)	Se (%)	Sp (%)	PPV (%)	NPV (%)
RDTM	1	0	19	180	0.6	100	100	9.55
MM	156	16	3	25	86.19	15.79	90.7	10.7

Keys: RDT = Rapid Diagnostic Test Method, MM = Microscopic method, Se = Sensitivity (TP/TP+FN) which is the fraction of those with the disease correctly identified as positive by the test. It is the probability that a sick individual will have a positive test, Sp = Specificity (TN/FN+TN) which is the fraction of those without the disease correctly identified as negative by the test. It is the probability that a well individual will have a negative test, PPV = Positive predictive value (TP/TP+FP) which is the fraction of people with positive tests who actually have the condition. The probability that those who have a positive test are really sick, NPV = Negative Predictive Value (TN/FN+TN) which is the fraction of people with negative tests who actually do not have the condition. It is the probability that those who have a negative test are really well, TP = Truly Positive, this is the test results that are positive for those who are well, FP = False Positive, this is the test results that are positive for those who are sick, TN =Truly Negative (The test results that are negative for those who are well), FN = False Negative, it is the test results that are negative for those who are sick, No = Number, % = Percentage

5. CONCLUSION

Plasmodium falciparum malaria infection exist among young adults of Babcock University with a prevalence rate of 0.5% and 86.0%, as detected by RDT and microscopic method, respectively and microscopic method appears to be a more reliable diagnostic tool for malaria infection than the rapid diagnostic method with a sensitivity of 86.19%, affirming it as the gold standard method for the diagnosis of malaria. In view of the low performance of the RDT in this study, we strongly suggest the potential modification/ improvement of the RDTs by future researchers. In addition, all RDT negative cases should be confirmed with microscopy to totally rule out malaria infection. However; laboratory personnel should be well trained to ensure proper reporting and interpretation of results. Accurate and proper diagnosis of malaria infection must be ensured before drug administration to prevent misdiagnosis, over diagnosis and over treatment of malaria cases that can make the *P. falciparum* parasite develop resistance to the few available potent anti-malarial drugs. To halt the cycle of *P. falciparum* malaria infection among the student population, we therefore recommend that the University authority should ensure the following control and preventive measures among others: fixing of bad window nets, provision of door nets at the entrance of the hostel rooms, availability of treated mosquito nets, replacement of torn mosquito nets, clearing of all mosquito breeding sites around the hall of residence, regular screening and treatment of all positive cases.

CONSENT

All authors declare that 'written' informed consent was obtained from the participants with assurance of anonymity and confidentiality before the commencement of the study.

ETHICAL APPROVAL

Ethical approval for the study was obtained from the Babcock University Health Research Ethics Committee (BUHREC), Babcock University, Ilishan-Remo, Ogun State, Nigeria, with the ethical approval registration number: BUHREC270/18.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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