The efficacy of rapid diagnostic test (rdt) in diagnosing *Plasmodium Falciparum* malaria in some selected health facilities in the cape coast metropolis of Ghana

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ABSTRACT

Malaria remains the highest cause of morbidity and mortality in Ghana and the rest of sub-Saharan Africa. Early diagnosis is therefore essential to treatment and eradication of the disease. This study was therefore designed to find out the efficacy of the First Response PfHRP2 malaria antigen test kit which promises to give reliable test results in diagnosing P. falciparum malaria as microscopy, the gold standard, since most of the malaria cases in our part of the world is caused by Plasmodium falciparum. The survey was carried out on a total number of 354 patients on who clinicians had requested for malaria test in four selected health facilities within the Cape Coast metropolis of Ghana. For each of these patients, both thick and thin blood films for microscopy, and rapid diagnostic test (RDT) were done using blood obtained from a finger prick or venipuncture. Of the 354 patients, 66 (18.6%) positive malaria cases were recorded for both microscopy and RDT. Fifty two (52) patients were diagnosed as truly positive for P. falciparum malaria by the rapid diagnostic test and 278 (78.5%) were diagnosed as truly negative. Paired sample T-test showed a p-value of 0.85 at α= 0.05 indicating no significant difference between microscopy and the RDT in the diagnosis of Plasmodium falciparum malaria. A prevalence rate of 18.6% was recorded for malaria cases with 78.8% occurrence of Plasmodium falciparum. The RDT recorded sensitivity and specificity of 79% and 95% respectively. The study lends credence to the fact that the rapid diagnostic test kit has comparable level of accuracy with microscopy and hence can be used in rapid screening of malaria but not as a complete substitute for microscopy in malaria diagnosis.

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INTRODUCTION

Malaria is very endemic in Africa and Cape Coast in the Central Region of Ghana is of no exception [1]. World Health Organisation reports estimate that about 3.3 billion people worldwide are at risk of the disease. Out of this number, 300 million to 500 million suffer the

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disease annually and 1 million to 3 million people die of which African children especially those in sub-Saharan Africa constitute about 90% [2]. Late and inefficient diagnosis of malaria largely caused by *P. falciparum,* in our part of the world, are factors that account for the increasing morbidity, mortality, drug resistance and the associated economic losses. There is therefore the need for an express and accurate diagnostic method for malaria which will help minimize and/or eradicate the disease and its allied effects.

The 2009 World Malaria Report estimates 8.3 million *P. falciparum* malaria cases in Ghana between years 2000 and 2007 and 3.2 million cases in year 2008. The report has it that there was no evidence of reduction in the number of cases between 2001 and 2007 and the number of in-patient reported cases as well as deaths resulting from malaria [2]. Reports from the MOH's (Ministry of Health, Ghana) *"Roll Back Malaria Strategic Plan 2001-2010,"* indicate that malaria is hyperendemic in Ghana with a crude parasite rate ranging from 10% to 70% and *P. Falciparum* is the major malaria parasite dominating [3]. *P. falciparum* malaria is the number one cause of morbidity, accounting for 40% of outpatient attendance with annual reported cases of 2.2 million between 1995 and 2001, with over 10% ending up on admission [4]. Early, proper healthcare is known to save life. As such an early and efficient method of diagnosing *P. falciparum* malaria will help in effective treatment which will help reduce morbidity and mortality that present with *P. falciparum* malaria. This study therefore seeks to find out the efficacy of the First Response PfHRP2 malaria antigen test kit which promises to give reliable test results in diagnosing *P. falciparum* malaria as microscopy, the gold standard, since most of the malaria cases in our part of the world is caused by *Plasmodium falciparum*.

MATERIALS AND METHODS

Study Design
This research was conducted in the clinical laboratories of the under listed health facilities in the Cape Coast metropolis.

1.   Central Regional Hospital.
2.   Adisadel Urban Health Centre.
3.   Ewim Urban Health Centre.
4.   Microclinics.

Materials used for this survey included the First Response PfHRP2 malaria antigen RDT kits, sterile lancets, alcohol swabs, and pipettes manufactured by Premier Medical Corporation Limited in India, microscope, microscope slides, latex gloves, Giemsa stain and patients who attended hospital within March 2011 at the various health facilities stated above. The entry criterion for the main trial was based on a clinician's request for a malaria test in a patient of any age and sex. Patients who were recruited for the study were those who reported at the out-patients department (O.P.D.), those who had been detained or admitted as in-patients, and ante-natal care (ANC) clients. Patients with a clinician's request for malaria test were invited to take part. Those who on their own or their guardians gave consent were then subjected to diagnosis by rapid diagnostic test and blood slide microscopy. Some information was taken from these patients through an interview and these included whether they had experienced any symptoms of malaria since the year begun, whether any form of malaria chemotherapy was given regardless of

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what medicine was given and whether they bought the drug with or without a clinician's prescription. The sample size required for the research was 354 with 95% confidence level and a 5% (i.e. α=0.05) margin of error. The sample size was calculated based on the formula:

$$n= \frac{t^2 \times p \times (1\text{-}p)}{m^2}$$

Where:

n→ sample size

t→ confidence level of 95% (standard value of 1.96)

m→ margin of error of 5% (standard value of 0.05)

p→ estimated prevalence of malaria in project area based on data obtained for malaria cases in 2010 [5].

One hundred and six (106) samples were taken from the Central Regional Hospital consisting of 68 females (64.2%) and 38 males (35.8%). One hundred and sixteen (116) samples, consisting of 83 females (71.6%) and 33 males (28.4%) were obtained from the Adisadel Urban Health Centre. Ninety two (92) samples were taken from the Ewim Urban Health Centre and this consisted of 75 females (81.5%) and 17 males (18.5%). Forty (40) samples which comprised 27 females (67.5%) and 13 males (32.5%) were obtained from Microclinics. There were no ethical matters concerned with this study, as results from routine laboratory diagnosis of clinical samples constituted the data for analysis; no particular identifiable group of patients were involved and their individual identities could not be traced.

Diagnosis of Malaria

Each patient for whom a clinician had requested parasitologic test for malaria and had consented to partake in the research was subjected to screening using both routine microscopy for visible parasites in the microscopic field and First response PfHRP-II malaria RDT kit for the detection of histidine-rich protein II antigen which is characteristic of *P. falciparum*. The slides and RDT kit used for each participant were given the same labels.

Blood Sample Collection

Blood sample collection as well as procedures involved in conducting microscopic tests to detect malaria parasites were done according to the routine procedures of the respective health facilities. Peripheral blood was drawn by pricking a finger with a lancet which is reported to be the most ideal because the density of developed trophozoites or schizonts is greater in blood from this capillary-rich area [6]. Both thick and thin blood films were prepared in the clinical laboratories of all the health facilities for microscopy. Thus, two blood slides were prepared for each participant, with collection done using the routine practice of the laboratory as stated earlier. The thick film was done by placing a drop of blood in the centre of a microscope glass slide and using the corner of a clean apparatus such as a cover slide to spread the blood to cover an area of about 10mm^2. The slides were labelled using the respective patient numbers assigned at the O.P.D. The slides were air-dried and were stained with Giemsa's stain for 20 minutes. The slides were then rinsed under mild running tap water and allowed to air-dry.

Speciation of the various *Plasmodium* species necessitated the preparation of the thin blood film since the survey was mainly concerned with *P. falciparum*. Thin blood films allow species identification since the parasite's appearance is best preserved in this preparation. That is, the fixed monolayer of red blood cells available in this procedure makes the morphological identification of the parasite to the species level much easier, hence provides greater specificity than the thick-film examination [7].

 The thin blood film was prepared by placing the smooth edge of a spreader slide in the drop of blood, adjusting the angle between slide and spreader to 45°, then allowing the blood to spread along the entire width of the spreader slide and gently smearing the blood with a swift and steady sweep along the surface. The film was then allowed to air dry and was fixed with methanol before staining.

Microscopic Examination

The thin and thick blood film slides were then observed under high-power optical microscope for the presence of malaria parasites. One hundred high power fields of thick and thin films, under oil immersion, were viewed for each slide at a $\times 1000$ magnification for 5 minutes or more before a slide was said to be positive or negative. For the sake of accuracy, services of very experienced clinical staff were deployed most especially in diagnosis using microscopy so that very much reliable results will be obtained. Slides were re-examined, especially the thin films, by expert microscopists to ascertain the presence of the malaria parasites and which species of *Plasmodium* was causing the infection. The presence of malaria parasites was determined and reported using the plus sign scheme. Speciation was done to differentiate the other *Plasmodium* species from *Plasmodium falciparum*. However, the other *Plasmodium* species were not classified.

Screening with RDT

The First Response RDT kit used is specific for *P. falciparum* and has a test band along its length impregnated with monoclonal antibodies which are specific for the HRP2 antigen for *P. falciparum*. The test was conducted according to the specifications provided by the manufacturer. Both positive and negative controls were set for each box of the kit that was used to be sure of the viability of the pieces of cassettes in each box. About 5μl of whole blood was taken for the RDT for each participant with a label on the kit as stated earlier. The blood sample was added into the sample well after which two drops (60μl) of assay buffer was added into the buffer well and the results read in 20 minutes at room temperature. A positive reaction was identified by the presence of two rose-pink colour bands at the control (C) and test (T) labels. A visible rose-pink label at the control (C) label only was indicative of a negative reaction. Absence of rose-pink colour at both control and test labels indicated an invalid result but none was recorded in the course of the research.

Data Analysis

The efficacy of the RDT was expressed by comparing the results of the RDT to that of microscopy. Data were entered, validated and analyzed using SPSS software (Version 16) and methods used by [10, 11, 12,]. Based on the under listed parameters, sensitivity and specificity were calculated for the RDT with regards to the values of the parameters obtained from the research.

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True Positives (TP): People with the disease and tested positive.
False Negatives (FN): People with the disease but tested negative.
True Negatives (TN): People without the disease and tested negative.
False Positives (FP): People without the disease but tested positive.
Sensitivity= TP÷ (TP+FN)
Specificity= TN÷ (TN+FP)

RESULTS

Three hundred and fifty four (354) patients were recruited for this study. Valid data, in the scope of the study, on all 354 participants were obtained. One hundred and one (101) of the participants, representing 29% were males and 253 which constitute 71% of the total sample size were females. The ages of the study participants ranged from 1 day old to 97 years as indicated on Table 1 below.

Table 1: Age Group Distribution of Respondents with Gender Composition

Age group	Frequency	Percentage	Male	Female
0-9	79	22.3	40	39
10-19	46	13	14	32
20-29	111	31.4	21	90
30-39	44	12.4	5	39
40-49	32	9	10	22
50-59	17	4.8	3	14
60-69	11	3.1	5	6
70-79	11	3.1	1	10
80-89	1	0.3	0	1
90-99	2	0.6	2	0
Total	354	100	101	253

Giemsa-stain microscopy detected 66 (18.6%) positive and 288 (81.4%) negative cases whereas the First response PfHRP2 malaria antigen detection test showed 67 (18.9%) positive and 287 (81.1%) negative cases. Out of the 67 positive cases detected by the RDT, 15 of them were negative for microscopy. Again, of the 287 patients who tested negative for the RDT on examination, 14 were positive for microscopy these were shown by the thin blood film to be infections of the other *Plasmodium* species other than *Plasmodium falciparum*.

Table 2: Results for the First Response PfHRP2 Card Test using Microscopy as the Test Reference

	Microscopy Results		
	Positive	Negative	Total
RDT Results			
Positive	52	15	67
Negative	14	273	287
Total	66	288	354

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The RDT results for the 354 patients were compared to the microscopic examination of blood smears which is the gold standard for malaria diagnosis to obtain the parameters for calculating sensitivity and specificity. The results are shown in Table 2.

The RDT recorded a sensitivity of 79% and a specificity of 95%. The 66 total positive malaria cases therefore yielded a malaria prevalence rate of 18.6% for the 354 total sample size that was considered. *Plasmodium falciparum* accounted for 61 of these 66 positive malaria cases. Occurrence of *Plasmodium falciparum* was therefore found out to be 78.8% and that of the other *Plasmodium* species was 21.2%.

An interview with the 354 participants revealed that 172 (49%) had experienced some symptoms within the previous months (i.e. January and February 2011) which they perceived as symptoms of malaria. One hundred and twenty four (124), representing 72.1%, of the 172 participants, took some form of medication whereas 46 (27%) were not on chemotherapy. Among the 124 participants who took some drugs on experiencing the symptoms, 86 (69.4%) were given drug prescription by clinicians whereas 38 (30.6%) bought drugs, without the authorization of any clinician, from pharmacy shops and other small scale "drug stores" in their localities. Seventy four (74) pregnant women were part of the 354 total samples on which the survey was conducted. Forty three (43) of the 74 pregnant women responded yes to have experienced some malaria-like symptoms in the previous months. Thirty four (34) of the 43 pregnant women took some form of medication. Out of the 34 who took medication, 25 took drugs prescribed by clinicians and 9 took over-the-counter drugs. The data is presented on Table 3.

Table 3: Results of Interview with Respondents

		Number of respondence	Male	Female
With malaria-like symptoms		172	43	86+ (43)*
Took drugs.		124	29	61+ (34)*
Place of drug prescription.	Hospital	86	16	45+(25)*
	Pharmacy	38	13	16+(9)*

*Pregnant women

The positive cases detected by both microscopy and the RDT were related to the age groups to find out the number of positive and negative cases recorded within each age groups. The results are shown in Table 4 below.

The positive RDT results were also related to the age groups to determine occurrence of *P. falciparum* within the age groups as well as determine if there was any significance in the ability of the RDT in diagnosing *P. falciparum* infection among the age groups.

As presented on Table 5, age group 0-9 recorded the highest infection with 21 individuals with age groups 50-59, 70-79, 80-89 and 90-99 recording the least infection.

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Table 4: Positive Results for RDT in Relation to Age Group of

Age Group	RDT Results		Total
	Positive cases	Negative cases	
0-9	16	63	79
10-19	10	36	46
20-29	13	98	111
30-39	2	42	44
40-49	5	27	32
50-59	1	16	17
60-69	3	8	11
70-79	1	10	11
80-89	1	0	1
90-99	0	2	2
Total	52	302	354

Table 5: Occurrence of Malaria Infection among the Age Groups based on RDT Results

Age Group	Cases	Occurrence (%)
0-9	21	31.3
10-19	15	22.4
20-29	16	23.8
30-39	3	4.5
40-49	5	7.5
50-59	1	1.5
60-69	3	4.5
70-79	1	1.5
80-89	1	1.5
90-99	1	1.5
Total	67	100

DISCUSSION

Malaria is very endemic in Africa and accounts largely for the high incidence of morbidity and mortality in Africa. Different species of the genus *Plasmodium* have been implicated in malaria infection in our part of the world. However, *Plasmodium falciparum* has shown to be the most prevalent in our locality. The microscopic diagnosis of this parasite is so labourious, time-consuming and requires so much expertise. The research therefore sought to test the efficacy of the First Response PfHRP2 malaria antigen test kit to find out if it has comparable performance as microscopy and if it is reliable in the diagnosis of *P. falciparum*.

A paired sample T-test for the microscopy and RDT results recorded a p-value of 0.85 at $\alpha= 0.05$ indicating no significant difference in the potential of the RDT and microscopy to diagnose the presence or absence of *P. falciparum* infection. Again, a test statistic value of 0.73 for Pearson

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correlation analysis at α= 0.01 was recorded. This indicates that there is a strong positive correlation between the RDT and microscopy regarding their ability to diagnose *P. falciparum* malaria, hence supports the outcome of the paired sample T-test. With these it can be said that the RDT and microscopy showed no significant difference in their ability to diagnose a patient of the presence or absence of *P. falciparum*. Hence, the RDT is reliable for use in diagnosing malaria induced by *P. falciparum*.

The 66 positive cases of malaria infection among the 354 patients gave a malaria prevalence rate of 18.6%. Clinically diagnosed malaria accounts for about 25-40% (average 30%) of all outpatient clinic visits in all malaria-endemic countries in Africa [8]. In these same countries, between 20% and 50% of all hospital admissions are a consequence of malaria. This indicates that the 18.6% prevalence rate of malaria among the 354 samples used for the survey is relatively low. This could be due to the fact that Ministry of Health in Ghana has introduced a programme which seeks to promote self medication by subsidizing over the counter malaria drugs, although self medication has its own problems.

From the results, it can be observed that between the age groups, age group 0-9 recorded relatively the highest occurrence of 31.3%, meaning more individuals within that group were positive for malaria suggesting that people in this age group are more vulnerable to malaria infection [2]. That is, age group 0-9 constitutes children and children are known to have weak immune systems which make them very much susceptible to infections such as malaria.

Occurrence of *P.falciparum* was found to be 78.8%. This indicates that *P. falciparum* is really the most virulent among the parasites in the genus *Plasmodium* in our part of the world since the survey proves that it causes more than three-quarter of all malaria infections. The other species of *Plasmodium* are therefore implicated in only few cases of malaria infection. This finding therefore makes the use of the rapid diagnostic test (RDT) kit relevant since it is specific for diagnosing the most endemic *Plasmodium* species in our locality.

The RDT detected 67 positive cases and 287 negative cases. Fifteen (15) of the positive RDT results proved negative for microscopy. This inconsistency in the results might have arisen due to the persistence of the HRP2 antigen in blood without the parasite and possible parasite sequestration.

The RDT recorded a sensitivity of 79% and a specificity of 95%. In a similar experiment, they reported 93% sensitivity and 95% specificity for the RDT which they used in diagnosing malaria caused by *P. Falciparum* [9]. Reasonably high sensitivity and very high specificity were recorded for the RDT in this survey and can therefore give reliable diagnostic result.

Pearson correlation test showed that there is a weak correlation in the ability of the RDT to diagnose *P. falciparum* malaria among the different age groups. This result was obtained when the RDT result was related to the age group of the participants. The Pearson correlation coefficient (r) obtained was 0.036 at α= 0.05.

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