Evaluation of Paracheck-*Pf*™ rapid malaria diagnostic test for the diagnosis of malaria among HIV-positive patients in Ibadan, south-western Nigeria

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Febrile illnesses occur frequently among HIV positive patients and these are often treated presumptively as malaria in endemic areas. Parasite-based diagnosis of malaria will eliminate unnecessary treatment, reduce drug–drug interactions and the chances for the emergence of drug resistant *Plasmodium*.

We evaluated finger prick blood samples from 387 people living with HIV (PLWHIV) and suspected of having malaria by expert microscopy and Paracheck-*Pf*™ – a histidine-rich protein-II based malaria rapid diagnostic test. The study was conducted at the PEPFAR supported AIDS Prevention Initiative in Nigeria (APIN) Clinic of the University College Hospital Ibadan, southwest Nigeria. Outcome parameters were prevalence of malaria parasitemia, sensitivity and specificity of Paracheck-*Pf* as well as the positive and negative predictive values for Paracheck-*Pf* using microscopy of Giemsa-stained blood film as gold standard.

Malaria parasites were detected in 19.1% (74/387) of enrollees by microscopy and 19.3% (74/383) by Paracheck-*Pf*. Geometric mean parasite density was 501/μl (range 39–749 202/μl). Sensitivity and specificity of Paracheck-*Pf* at all parasite densities were 55.4% and 89.3% while corresponding figures at parasite densities ≥200/μl were 90.9% and 90.3%. Sensitivity and specificity at parasite densities ≥500/μl was 97.6% and 90.3%. Positive and negative predictive values for parasite density ≥200/μl were 55.4% and 98.7%, respectively.

Paracheck-*pf* was found to be a useful malaria diagnostic tool at parasite densities ≥200/μl facilitating appropriate clinical management.

Keywords: Adult, HIV, Malaria, Paracheck-RDT

Background

Human Immunodeficiency Virus (HIV) infection remains a global public health problem with over 34 million people living with HIV in 2010 and about 2.7 million newly acquired/diagnosed cases annually.[1] The bulk of the burden of HIV is in sub-Saharan Africa where malaria is endemic. The immune suppression that accompanies HIV naturally increases the susceptibility of HIV +ve persons to malaria.[2,3] Not only do HIV positive persons have more frequent attacks of malaria, the infection appears to be more severe in them.[4–7] In malaria endemic areas, febrile illnesses are often assumed to be due to malaria and are usually treated as such without recourse to laboratory confirmation. Microscopy of Giemsa-stained blood smear, which remains the gold standard, is laborious and requires electricity to power the microscope and trained personnel, all of which are often not available in malaria endemic areas. Malaria has no pathognomonic signs or symptoms and presumptive treatment of malaria often leads to over-diagnosis of malaria and unnecessary treatment with antimalarial drugs.[8] This practice carries with it numerous other problems, especially in HIV +ve persons, who are prone to many other infections such as

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DOI 10.1179/2047773213Y.0000000077

bacterial, mycobacterial, viral, fungal and other parasitic infections[9–11] which could present with fever. Apart from the delay in appropriate diagnosis of non-malaria febrile illness and consequent progression of the disease process, unnecessary treatment with antimalarial drugs increases the already high pill burden of HIV patients who are on antiretroviral and/or opportunistic infection prophylaxis,[12] with the attendant risk of drug–drug interaction. Poor drug compliance (secondary to high pill burden) and emergence of drug resistant plasmodial or retroviral strains are some other possible risks.

These challenges underscore the need for accurate, inexpensive, simple to use, and rapid malaria diagnostic tests. Histidine-rich protein-II (HRP-II) based malaria rapid diagnostic tests (RDTs) have been recommended as good options.[13] Histidine-rich protein-II based RDTs are more robust in that they can withstand the temperature fluctuations of tropical malaria endemic regions better than the enzyme-based RDTs, parasite lactate dehydrogenase (pLDH) and aldolase, which mandatorily require air-conditioned storage[14,15] conditions that cannot be guaranteed in sub-Saharan Africa where the bulk of the malaria burden is.

Although Paracheck-*Pf*™ has already been shown to be an effective tool for malaria diagnosis in many populations of children and adults in previous studies[16–19] we nonetheless believe that it is important to evaluate its sensitivity and specificity among HIV positive persons especially in an area of high malaria transmission. In addition, some malaria RDTs have been shown to detect sub-microscopic infections.[20,21] We report here the performance of Paracheck-*Pf*, an HRP-II based malaria RDT (Paracheck-*Pf* RDT), in the detection of *Plasmodium falciparum* in capillary blood samples of HIV +ve patients suspected of having malaria at the adult ARV out-patient clinic of the University College Hospital, Ibadan, south-western Nigeria, where malaria is endemic. This study was triggered by the routine prescription of antimalarial drugs for patients attending the clinic which is often based on presumptive diagnosis.

Subject and Methods

Study site

The study was conducted at the HARVARD partnered President's Emergency Plan for AIDS Relief (PEPFAR) funded APIN adult ARV out-patient clinic, University College Hospital, Ibadan in south-western Nigeria. Ibadan lies at about 7°23′16″ latitude and 3°53′47″ longitude coordinates, with precipitation ranging from 24 to 178 mm/month and temperatures of 21.5–34.8°C in average conditions.[22] Ibadan is located 160 km from the Atlantic coast and has an average elevation of 200 m. The human population of Ibadan is estimated to be about four million. Malaria transmission is hyper-endemic in Nigeria, occurring throughout the year with a major peak during the rainy season months of May to October and a lower peak in the dry season months of November to April.[23] The prevalence of HIV sero-positivity in Nigeria is 3.9% [UNGASS (2010), 'UNGASS Country Progress Report: Nigeria'].

Nigeria changed its malaria treatment guidelines in January 2005 in line with the World Health Organization recommendation from chloroquine to artemisinin-based combination therapy (ACT) with a preference for artemether–lumefantrine or artesunate–amodiaquine in that order.[24] The standard of care for malaria at the PEPFAR clinic of the University College Hospital is ACT. Both artemether–lumefantrine and artesunate–amodiaquine are available on prescription at no cost to patients who receive care at the PEPFAR clinic. However, antimalarial drugs ranging from chloroquine to ACTs are available as over-the-counter drugs in private drug stores in Nigeria. The PEPFAR clinic in University College Hospital is the main facility in the city of Ibadan where persons living with HIV (PLWHIV) receive care for HIV infection and other health needs. The facility runs an out-patient clinic five days a week and in-patient services seven days a week. There are 10 medical officers dedicated to the clinic and a large pool of specialists within the hospital to draw on for care. Services rendered include walk-in clinics, confirmatory western blot testing for HIV, high quality counseling unit, diagnostic and follow up viral load measurement, CD4 T-cell counts, and drug pick-ups.

Baseline insecticide-treated net (ITN) usage in south west Nigeria is 1–20.1% in pregnant women[25,26] and 1.7–26.6% among the under-five-year-olds.[27,28] Participants in this study had received ITNs whenever the Federal Ministry of Health made ITN available. We estimate ITN ownership to be about 40% among patients attending the PEPFAR clinic at the University College Hospital Ibadan where this study was conducted. Participants also receive cotrimoxazole prophylaxis if their T-cell lymphocyte count was <350 cells/mm^3.

The University of Ibadan/University College Hospital Institutional Review Committee provided ethical approval for the study. A signed informed consent was also obtained from each study participant before enrollment.

Study design and study procedures

Clinical evaluation

In a prospective study, 387 PLWHIV who were registered and received care at the PEPFAR clinic of the University College Hospital were enrolled between October 2009 and September 2010. Convenience

sampling method was used in a prospective, descriptive study design in which every consecutive patient referred to the study team by attending physicians, if their symptoms were considered suggestive of malaria, were enrolled. Patients were enrolled if they had symptoms suggestive of acute uncomplicated malaria, were 18 years and above, and provided a written, informed consent. Patients who refused to provide informed consent or had clinical features of severe malaria were exempted. Following enrollment, an interviewer-administered questionnaire was used to obtain information on patient's socio-economic and demographic background, history of arthritis, medication history especially antimalarial therapy within two weeks of enrollment, and history of blood transfusion within the same time frame. Presenting symptoms and signs of current illness were recorded. Also recorded were CD4 counts and viral load within six months of enrollment into the study as well as the use of opportunistic infection prophylaxis. Clinical measurements included weight, pulse rate, and temperature of each study participant. Thereafter, thick blood smears were prepared from a finger prick for microscopy. Blood was also obtained from the same finger prick for Paracheck-*Pf* RDT testing and hematocrit evaluation.

Preparation of blood smears and microscopic examination

Thick blood smears were prepared from a finger prick aseptically. Blood smears were subsequently dried and stained using standard procedures. Prepared blood smears were then examined by two experienced microscopists using a light microscope at $\times 1000$ magnification for the presence and quantification of malaria parasites. The microscopists who were blinded to the results of Paracheck-*Pf* assay screened each smear independently, with the mean of the two counts per patient recorded as that enrollee's parasite count. Discordant readings were resolved by one of the senior investigators (COF). The definitive count of asexual parasites was calculated assuming a total white cell count of 8000/mm^3. A blood smear was only declared free of parasites after examining 100 high power fields. Qualitative results of malaria microscopy was made available within three hours of enrollment and all smear positive patients received a six-dose regimen of artemether–lumefantrine, which is the standard of care in Nigeria.

Parasite detection using Paracheck-Pf and determination of hematocrit

Paracheck-*Pf* [Orchid Biomedical Systems, Goa, India] was used for detection of *P. falciparum* according to the manufacturer's instructions. Staining of both the control and test bands (irrespective of the intensity of staining) was taken as a positive result. Staining of only the control band was considered indicative of a negative result whereas the test was considered invalid if the internal control band was not stained.

Capillary tubes were filled up to the mark with blood from the same finger prick used for preparation of blood smears and Paracheck-*Pf* test. The capillary samples were spun in a Hawksley^{TM} micro-hematocrit centrifuge and read using a Hawksley reader.

Data analysis

All patient information and test results were entered into a computer and analysed using SPSS Version 15 (SPSS Inc. Chicago, IL, USA). Frequency tables were generated for relevant variables. Descriptive statistics such as means±standard deviations were used to summarize quantitative variables while categorical variables were summarized with proportions. The chi-squared test was used to investigate associations between two qualitative variables. Analysis of variance was used to compare the mean values of more than two groups. All analyses were done at the 5% level of significance. Outcome parameters were prevalence of malaria parasitemia, sensitivity, and specificity of Paracheck-*Pf* as well as the positive and negative predictive values for Paracheck-*Pf*. With the corresponding results of the microscopy taken as the 'gold standard', the sensitivity and specificity of Paracheck-*Pf* were calculated as TP/(TP+FN) and TN/(TN+FP), respectively. Positive predictive values (PPV) were calculated as TP/(TP+FP) and negative predictive values (NPV) as TN/(TN+FN), where TP = True positive, TN = True negative, FP = false positive and FN = False negative.

Results

Demographic characteristics

Three hundred and eighty-seven HIV positive adults with clinical features suspected to be malaria were enrolled at the PEPFAR clinic at the University College Hospital, Ibadan, Nigeria between October 2009 and September 2010. The ages of the study participants ranged from 18–70 years with a mean of 36.7 years±9.5. Three hundred and twenty-two of the 386 (83.2%) enrollees who provided their ages were aged 45 years or less. A large majority (320; 82.7%) of the study participants were females, of which 52 (16.3%) were pregnant. The population of patients attending the PEPFAR clinic at the University College Hospital was about 16 000 with a 33% male, 67% female distribution at the time of the study. Almost 8000 patients were receiving ART in the study center at the time of the study. About two-thirds (246/387; 65.1%) of the study participants had received at least secondary school education while 326/387 (84.2%) were gainfully employed. Enquiries into the marital status of the study participants revealed that 73.5% (275/374) were married. Further details of the demographic characteristics of enrollees are shown in Table 1.

Clinical features of study participants

One hundred and forty-five of 387 (38%) participants had CD4 count $\leqslant$250 cells/mm^3, 267/354 (69%) were on antiretroviral therapy while 174/387 (45.7%) were on daily cotrimoxazole prophylaxis. Thirty-three (8.5%) study participants were not on cotrimoxazole or antiretroviral therapy. Fever or a history of fever (71.6%) within 24 hours of enrollment was the most common presenting complaint among study participants. The four next most frequent presenting complaints among enrollees were headache (58.9%), anorexia (50.1%), aches and pains (49.9%), and chills and rigors (38.8%). Presenting symptoms occurring in more than 5% of the study participants are shown in Table 2. About half (191/378; 50.5%) of the study participants had an axillary temperature of 37.5°C or above while 52.7% (197/374) were anemic (hematocrit <33%).

About a third (124/387; 32.0%) of the study participants reported having taken antimalarial drugs in the two weeks preceding enrollment. Seventeen (13.7%) of them had taken more than one course/type of antimalarial drugs. Sulfadoxine–pyrimethamine was the most frequently used antimalarial drug (55/124; 44.4%) followed by chloroquine 28 (22.6%), and various ACTs [artemether–lumefantrine (17), artesunate–amodiaquine (7) and others (4)]. Twenty-six (21%) study participants who took antimalarial drugs had a history of artemisinin monotherapy use while eight (6.5%) had taken amodiaquine monotherapy. Only four of the 55 patients who had a history of sulfadoxine–pyrimethamine use were pregnant.

Results of malaria screening by microscopy

Malaria parasite was detected in 19.1% (74/387) of enrolled patients by microscopy of Giemsa-stained thick blood smear. The parasite density ranged from 39/µl to 749 206/µl with a geometric mean parasite density of 501/µl. Thirty-nine of 74 patients (52.7%)

Table 1 Socio-demographic characteristics of HIV +ve patients suspected of having malaria enrolled into the Paracheck-*Pf* in the diagnosis of malaria

Characteristics	Number (%)
Sex (*N* = 387)	
Males	67 (17.3)
Females	320 (82.7)
Level of education (*N* = 378)	
None	39 (10.3)
Primary/Qur'anic	93 (24.6)
Secondary	142 (37.6)
Post-secondary	104 (27.5)
Occupation (*N* = 377)	
Student/unemployed/retired	51 (13.5)
Petty trader	113 (30.0)
Primary school teacher/junior civil servant/artisan/transporter	125 (33.2)
High school teacher/middle level civil servant/middle business person	70 (18.6)
Major business person/professional	18 (4.8)
Marital status (*N* = 374)	
Single	49 (13.1)
Married – monogamy	192 (51.3)
Married – polygamy	83 (22.2)
Separated	15 (3.9)
Lives with partner but not married	3 (0.8)
Divorced	6 (1.6)
Widowed	26 (7.0)

Table 2 Presenting symptoms occurring in over 5% of HIV +ve patients suspected of having malaria enrolled and result of malaria microscopy

Presenting symptoms	Number (%) 387 (100)	MP +ve	MP −ve
Fever or a history of fever	277 (71.6)	58 (20.9)	219 (79.1)
Headache	228 (58.9)	44 (19.3)	180 (80.7)
Loss of appetite (anorexia)	194 (50.1)	40 (20.6)	154 (79.4)
Aches and pains	193 (49.9)	43 (22.2)	150 (77.3)
Chills and rigors	150 (38.8)	33 (22.0)	117 (78.0)
Cough	139 (35.9)	23 (16.5)	116 (83.5)
Sleeplessness	125 (32.3)	24 (19.2)	101 (80.8)
Abdominal pains	109 (28.2)	20 (18.3)	89 (81.7)
Vomiting	94 (24.3)	23 (24.5)	71 (75.5)
Irritability	59 (15.2)	12 (20.3)	47 (79.7)
Body weakness/tiredness	43 (11.1)	7 (16.3)	36 (83.7)
Catarrh (rhinorrhea)	33 (8.5)	3 (9.1)	30 (90.9)
Dizziness	23 (5.9)	8 (34.8)	15 (65.2)

MP = Malaria parasite.

and 46 (62.2%) of the 74 patients with patent parasitemia had parasite densities <200/µl and <500/µl, respectively, while 18/74 (24.3%) of them recorded parasite densities of 5000/µl and above. Although malaria parasitemia was more prevalent among female participants (20% vs 14.9%), the difference was not statistically significant ($P = 0.396$). The prevalence of malaria parasitemia was also not significantly affected by pregnancy status among the women, educational status, or occupation. None of the presenting symptoms were positively correlated with malaria parasitemia be it at all parasite density, ≥200/µl or ≥500/µl. There was no correlation between a positive history of antimalarial drug use within two weeks of enrollment and the result of malaria microscopy (Table 3). Malaria parasitemia was significantly less prevalent among patients receiving antiretroviral drugs but not Cotrimoxazole (Table 3). Patients whose CD4 counts were ≥250 cells/mm^3 were significantly less likely to have patent parasitemia. Although the prevalence of malaria parasitemia was higher during the rainy season than the dry season (34/153; 22.2% vs 40/234; 17.1%), the difference was not statistically significant. In the same manner, the geometric mean parasite density though higher (640/µl) during the high transmission rainy season than the dry season (406/µl) was not statistically significant.

Prevalence of malaria parasite by Paracheck-Pf

Paracheck-*Pf* malaria RDT detected malaria in 19.3% (74/383) of the enrollees. Four patient samples (1%) yielded indeterminate results on testing with Paracheck-*Pf*. Microscopy failed to detect malaria parasites in all four samples that gave indeterminate results. Further details of comparison of microscopy and Paracheck-*Pf* test results are shown in Table 4. It is noteworthy that none of the 14 enrollees who had a history of arthritis recorded false positive results on RDT testing. There was concordance between microscopy and RDT result in 317/387 cases (81.9%). There were 33 cases of false positive results and 11 (33.3%) of these had a history of recent antimalarial drug treatment within two weeks of enrollment. However, there was no correlation between a positive history of recent malaria treatment and results of RDT. Another 33 (8.5%) patients recorded false negative RDT results. Parasite density was less than 200/µl in 84.8% (28/33) of patients with false negative RDT results while parasite density was less than 500/µl in all but one [97% (32/33)] of the patients who tested false negative to RDT. One of the 33 patients with a false negative result had a parasite density of 20 400/µl. The overall sensitivity of Paracheck-*Pf* was found to be 55.4% at all parasite densities. This rose to 90.9% and 97.6% at parasite densities ≥200/µl and ≥500/µl, respectively. The specificity of Paracheck-*Pf* in this study was of 90.3% at parasite densities ≥200/µl and ≥500/µl.

Table 3 Correlation between microscopy and selected clinical characteristics of study participants

Characteristics	Microscopy positive	*P*-value
Malaria treatment within two weeks		
Yes	19/124 (15.3%)	0.264
No	52/256 (20.3%)	
Temperature (°C)		
Mean (±SD)	37.75±0.91	0.134
High temperature (≥37°C)		
Yes	44/191 (23%)	0.69
No	29/187 (15.5%)	
Packed cell volume (%)		
Mean (±SD)	31.27±5.7	0.914
Anemia (PCV<33%)		
Yes	39/197 (19.8%)	0.897
No	34/177 (19.2%)	
CD4+T-cell count		
≤250 cells/mm^3 (149)	35 (23. 5%)	0.043
>250 cells/mm^3 (233)	37 (15.9%)	
Antiretroviral treatment		
Yes (267)	44 (16.5%)	0.018
No (87)	21(24.1%)	
Cotrimoxazole treatment		
Yes (174)	26 (14.9%)	0.067
No (180)	39 (21.7%)	

Discussion

During this study we evaluated the prevalence of malaria parasitemia among HIV seropositive adult patients suspected of having malaria at the PEPFAR clinic in Ibadan, Nigeria by microscopy and Paracheck-*Pf* RDT. The prevalence of malaria parasitemia was only 19.1% by expert microscopy with a geometric mean parasite density of 501/µl. This is similar to the overall prevalence of 18.9% malaria parasitemia reported by Onyenekwe *et al.*[29] in a survey of asymptomatic HIV +ve persons in south-eastern Nigeria, an area of similar intensity of malaria transmission. The prevalence of malaria parasitemia among the study population is also similar to the 20.2% prevalence, which was obtained among 391 sero-negative blood donors in the same study hospital a few years earlier.[30]

The range of parasite densities was very wide ranging from 39/µl to 749 206/µl with over half of the patients recording parasite densities ⩽200/µl while almost two-thirds had parasite density less than 500/µl. The large proportion of patients with parasite density less than 200 asexual parasites/µl is reminiscent of the findings of an earlier study, which evaluated malaria parasitemia among healthy blood donors in the same hospital about three years before the current study in which about 76% of the parasitemic study participants recorded parasite densities ⩽200/µl.[30] Earlier workers have also reported low prevalence of malaria parasitemia among HIV positive persons receiving HAART and/or co-trimoxazole therapy.[31–33] Reports

of antimalarial properties of some antiretroviral drugs especially the protease inhibitors[34,35] probably contribute to the low prevalence of malaria parasite in HIV +ve persons on HAART. French *et al.*[4] had reported increased rates of malaria infection with deteriorating immune status. It is only reasonable to expect that the level of immune-competence will improve among patients on HAART as the CD4 count rises. This might translate to a partial if not complete restoration of the patients' earlier malaria semi-immune status.[36] About 70% (270/387; 69.8%) and 45.7% (177/387) of our study participants were receiving antiretroviral and co-trimoxazole, respectively.

The relatively low prevalence of malaria parasitemia in this group of HIV +ve patients who would have been diagnosed presumptively as having malaria and treated with an ACT further underscores the need for parasite based diagnosis of malaria. Given the low density of parasitemia in the majority of the patients, it is unlikely that the symptoms of over half of those who were parasitemic were due to malaria. Nakanjako *et al.*[32] working in Uganda, an area of low to moderate malaria endemicity reported similar low counts in their study during which they followed up asymptomatic malaria among PLWHIV on HAART. None of the patients in the series reported by Nakanjako *et al.*[32] with patent parasitemia reported signs and symptoms of malaria infection during the six months of follow up. Mills *et al.*[37] had also reported a markedly low incidence of malaria among HIV-positive adults who were receiving antiretroviral therapy, cotrimoxazole, and slept under ITN in rural Rakai, Uganda an area described as meso-endemic for malaria.

Performance of Paracheck-Pf

Paracheck-*Pf* RDT proved to be accurate and reliable for detecting malaria parasite densities of ≥200/μl in the population of HIV positive patients in this study. The finding that the sensitivity and specificity of Paracheck-*Pf* RDT increased with increasing parasite density is consistent with previous reports.[38–41] The recorded sensitivities of 90.9% and 97.6% at asexual parasite densities of ≥200/μl and ≥500/μl, respectively, means that the test will give less than nine false negative results for 100 cases of *P. falciparum* at a parasite density of 200/μl and above while it will fail to detect only 2.5% at a parasite density >500/μl microscopically confirmed. A further evidence for the good performance of Paracheck-*Pf* is the associated high negative predictive values of 98.7% and 99.7% at the two cut off parasite densities of 200/μl and 500/μl, respectively. While the high sensitivities allow the clinician to be confident of not missing practically any significant malaria cases, the high negative predictive values also allow the physician to confidently diagnose RDT-negative persons as non-malaria patients. This way, other causes of fever can be searched for and appropriate treatment given early. The high sensitivity and specificity obtained in this study is consistent with reports by previous workers.[17,19,42,43]

It is, however, a matter of concern that one of the patients with a false negative RDT result had a high parasite density of 20 400/μl. Previous workers have reported similar findings.[17,39,44,45] Discordance between microscopy and RDT results at such high parasite densities has been attributed to a '*prozone effect*'.[46] The prozone effect which is also known as high dose-hook phenomenon is defined as false negative or false low results in immunological reactions due to an excess of either antigens or antibodies. False negative results though generally associated with low parasite densities could be due to a number of other reasons. One of these reasons is that the *Plasmodium falciparum (Pf)* HRP-II protein has been reported to exhibit a high level of polymorphism.[47–49] Polymorphism in the genes encoding the HRP-II protein is an important factor that may affect the performance of RDT based on antigen detection. The presence of non-falciparum infection will also give a false negative result as only *P. falciparum* releases HRP-II. Non-falciparum malaria may therefore be misdiagnosed as malaria negative. Hence, in geographical regions where there is the possibility of having other species of *Plasmodia* causing malaria, Paracheck-*Pf* would give false negative results for malaria. In such locations, an RDT with a pan-malaria antibody will be a better option. Microscopy will also be useful in complementing the HRP-II only RDT test. The proportion that may be due to non-falciparum infections in our environment will however be quite low since over

Table 4 Comparison of Paracheck-*Pf* malaria rapid diagnostic test versus thick smear microscopy for detection of *Plasmodium falciparum* among adult HIV +ve patients with presumptive diagnosis of malaria

	Malaria microscopy results		
Paracheck-*Pf* result	Positive thick smear	Negative thick smear	Total
Positive	41 (10.6%)	33 (8.5%)	74 (19.1%)
Negative	33 (8.5%)	276 (71.3%)	309 (79.8%)
Indeterminate	0 (0%)	4 (1%)	4 (1%)
Total	74 (19.1%)	313 (80.9%	387 (100%)

95% of malaria infections in Nigeria are due to *P. falciparum*.[23] The limitation of the light microscope in detecting malaria parasite is also noteworthy and may be responsible for false negative RDT results even in expert hands.[19,20,50] Minja *et al.*[20] and Batwala *et al.*[50] reported that the HRP-2 RDTs detected some cases of sub-microscopic parasitemia which were confirmed by PCR, a more sensitive method for detection of malaria parasite.

The occurrence of false positive RDT results during this study was high (33/74; 44.6%). This is consistent with findings in areas of high malaria transmission and could be due to a number of reasons, which include presence of sub-patent malaria infection, recently treated malaria infection, and rheumatoid factor positivity.[17,39,44] In high transmission areas such as we have in south-western Nigeria, the prevalence of sub-patent malaria is high.[51] False positive results may thus occur because of the limitation in the sensitivity of malaria microscopy. Some of our study participants who showed a false positive result to RDT might have had a recent malaria infection, which had been successfully treated. Delayed clearance of HRP-II following acute malaria infections is well documented.[42,52,53] Histidine-rich protein-II based tests have been shown to remain positive for up to one month (mean time being about two weeks) after parasite clearance. Histidine-rich protein-II clearance time is a function of many factors that are not well understood. However, it has been linked to the patient's parasite density. The higher the parasitemia, the longer it takes the body to eliminate HRP-II, thus HRP-II antigenemia may persist for weeks after successful antimalarial therapy has eradicated the asexual blood stage parasites.[38,53] It is of note that almost a third of the enrollees had a history of antimalarial drug use within two weeks of enrollment. The intensity of transmission during seasonal changes has been documented to affect the level of sensitivity and specificity in some large studies.[17,39] Other co-morbidities such as rheumatoid factor heterophilic antibodies can also result in false-positive results. Although we did not test for rheumatoid factor during this study, it is, however, noteworthy that none of the 14 patients that had a history of arthritis recorded a false positive malaria RDT result.

In conclusion, the results from this study show that the performance of Paracheck-*Pf* was comparable to microscopy in the diagnosis of malaria in HIV +ve patients at parasite density $\geq$200/µl. This is similar to the findings of Mills *et al.*,[37] who evaluated the utility of Binax Now malaria RDT (an HRP-II plus pan malaria aldolase-based RDT) in Uganda. Paracheck-*Pf* and other malaria RDTs offer the opportunity to extend the benefits of parasite-based diagnosis beyond rural or primary health care level to a busy PEPFAR clinic in a tertiary healthcare facility by allowing prompt decision making in the confirmation or elimination of a diagnosis of malaria. Repeat RDT testing in symptomatic patients with low counts who test negative will become positive as parasite density rises even if they were missed initially as sensitivity and specificity of HRP-II-based malaria RDT increases at higher parasite density. In this regard it is important that patients with negative test results return to the health facility for repeat testing if their symptoms have not resolved within two days of the initial consultation. The emergence and widespread dissemination of drug resistant plasmodium infection which necessitated the switch from the cheap and easily available chloroquine to the more expensive ACT[15] has made RDTs more cost-effective than a decade ago.[54] In addition, RDTs are now very widely used requiring mass production with its attendant benefit of cost reduction contributing to the cost-effectiveness.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

We would like to thank our study participants, the medical and nursing staff of PEPFAR supported APIN Clinic University College Hospital for facilitating the study, and our research staff, especially Mrs Bola Akinyele, Mrs Fatima Abdulsalam, Miss Grace Egunyomi and Mr Tunji Agboola. We also thank Mr Funsho David for his participation during the early phase of the study. This study was partly funded by APIN Plus (PEPFAR) program.

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