

Original Research Article

Reliability of a rapid test for diagnosing malaria in people living with human immunodeficiency virus infection in Cameroon

ABSTRACT

Aims: This study aimed at appraising the performances of a RDT for diagnosis of malaria in people living with HIV infection.

Study design: This study was a cross-sectional study.

Place and Duration of Study: The study was carried out at the District hospital of Deido in Douala, Cameroon between August 2015 and March 2016.

Methodology: A total of 723 patients were included in the study and examined for malaria using light microscopy (Giemsa stained blood films) and RDT. Results were analyzed and compared using GM as the reference standard. HIV infection was confirmed using immunochromatographic- and ELISA-based methods. CD4 lymphocytes count was determined by flow cytometry.

Results: Overall, *Plasmodia* were found in 121 (16.7%; 95%CI: 14.2%-19.6%) and 91 (12.6%; 95%CI: 10.3%-15.3%) using light microscopy and RDT respectively. The sensitivity and specificity of RDT was 75.2% (95%CI: 66.8%-82.1%) and 100% (95%CI: 99.4%-100%) respectively. The positive and negative predictive value was 100% (95% CI: 95.9% -100%) and 95.3% (95% CI: 93.3% - 96.7%) respectively. The agreement between both methods was excellent ($\kappa = 0.835$; $P < .0001$).

Conclusion: The study showed a good performance of the RDT in terms of specificity, PPV, NPV, accuracy and agreement. This test might represent a good alternative to the standard method for diagnosis of malaria in PLVIH along with general population.

Keywords: *Malaria, Rapid diagnostic test, diagnosis performance, HIV-infected people, Cameroon.*

1. INTRODUCTION

Malaria stills a major public health concern through the world and Africa especially. According to the latest WHO estimates, this disease was responsible for 214 million and 438,000 deaths respectively. The burden is more important in Sub-Saharan Africa countries which account for 88% and 90% of all cases of morbidity and mortality [1].

Diagnosis is a key ingredient for sustainable and effective decreasing in malaria burden in terms of morbidity and mortality. Unfortunately, it relies on clinic in most of malaria endemic areas which are often resources-constrained and remote [2-3]. Besides, many authors reported poor performances of this diagnostic approach in terms of sensitivity and specificity [1, 4]. On the other hand, light microscopy is used as gold standard to confirm any clinical suspicion of malaria in most of healthcare facilities of these countries. This method is cheap and simple but labor intensive and time consuming and requires well-trained personnel especially for detection of low parasite densities [5].

These limitations are very important in order to avoid the unnecessary use of artemisinin-based combination therapies (ACTs), due to overdiagnosis, as this could lead to the development of drug

26 resistance [1]. Furthermore, a significant proportion of patients who need treatment could not promptly
 27 receive it or not at all. As a result, the risk of malaria-associated severe adverse effects and deaths
 28 might be increased in these patients. This matter takes sense when over- and underdiagnosis
 29 fractions are mainly important in groups at risks such as children, pregnant women along with
 30 immunocompromised people as those infected with HIV.

31 It looks critical to develop and implement new diagnosis alternative upon their evaluation in
 32 community especially in remote areas. Thus, this study aimed to appraise the diagnosis performances
 33 of a rapid diagnostic test (RDT) for malaria in HIV-infected people which are an important group at risk
 34 of malaria-related mortality.

35 **2. MATERIAL AND METHODS**

36 **2.1 Study site**

37 This study took place in the town of Douala (Littoral Region, Cameroon). Douala is located 3°48'N,
 38 10°08'E, near the Atlantic coast, within the Congo-Guinean phytogeographical zone characterized by
 39 a typical equatorial climate with two rainy seasons extending from March to June and from September
 40 to November [6]. The city is 1 m above sea level and receives over 3,500 mm rainfall annually. It is
 41 the main business city in Cameroon. These environment conditions are propitious for creation of
 42 breeding sites malaria vectors. Douala is a port city where many worse behaviors (liquors
 43 consumption, prostitution) significantly increase the risk of sexually-transmitted diseases (STDs) such
 44 as Human immunodeficiency virus infection. Douala is the main business one of the country and is
 45 ranked sixth (5.5%) in terms of HIV prevalence rate [7]. Participants were recruited at the district
 46 hospital of Deido which greets people coming from all parts of Cameroon owing to its strategic
 47 location and sustainable and constant supply with CD4 cells reagents and antiretroviral drugs for
 48 management of people living with HIV (PLWHIV).

49 **2.2 Study population**

50 The study population as consisted of individuals aged 5-49 years old of both sexes living with HIV and
 51 under HAART regimen. They were recruited in a convenient way and therefore a total of 723 patients
 52 were included in the study. Any patient who did not meet any of these abovementioned criteria were
 53 excluded from the study.

54 **2.3 Study design**

55 This cross-sectional and prospective study was carried out between August 2015 and March 2016.
 56 Participants included in the study were hospital-confirmed being HIV-infected, attending at the district
 57 hospital of Deido for routine control and willing to participate. Prior to their inclusion, they were given
 58 information, education and communication on malaria and HIV infection. Thereafter, an informed
 59 consent form was signed by each participant following explanation of objectives of the study to them.
 60 Approval of parents or guardians of children was also sought. A structured questionnaire was used to
 61 collect socio-demographic, clinic and biological data. Blood samples were collected and transported
 62 to the Laboratory of the district hospital of Deido for parasitological analyses. Investigative methods
 63 included a questionnaire approach, clinical and parasitological analyses.

64 **2.4 Blood collection**

65 About five milliliters (5 mL) were collected from each participant by venipuncture into sterile plastic
 66 syringes. Blood samples were then transferred into EDTA and dry tubes for all performing
 67 hematological, parasitological and biological analyses respectively. All tubes were labeled with the
 68 patients' barcode and pathology number. Blood samples were centrifuged at 3000 rpm for 3-5
 69 minutes and sera were stored at 2-8°C until analysis.

70 **2.5 Confirmation of HIV infection**

71 Prior to their inclusion in the study, the participants were tested for HIV infection for confirmation. HIV
 72 diagnosis was performed using Determine Alere HIV 1/2 rapid diagnosis test followed by
 73 Immunocomb HIV1/2 (Organics) accordance to the national guidelines. The former test is an immuno-
 74 chromatographic rapid test for qualitative detection of antibodies specific to HIV in human serum,
 75 plasma or whole blood. The latter is based on the enzyme-linked immunosorbent assay (ELISA)
 76 principle.

77 **2.6 CD4 cells count**

78 CD4+ T lymphocytes were counted with a flow cytometer CyFlow® (Sysmex-Partec Görlitz, Germany)
 79 according to the manufacturer's instructions. Briefly, 20 µL of phycoerythrin-conjugated monoclonal
 80 antibody to human CD4 (mAb PE MEM241, Partec GmbH, Görlitz, Germany) were slightly mixed with
 81 20 µL of whole blood into a test tube and incubated for 15 minutes at room temperature, protected
 82 from light. Then, 800 µL of no-lyse buffer were added to the mixture. After homogenizing the content,
 83 the tube was introduced into the CyFlow Counter for automatic counting [8]. The CD4 results were
 84 grouped into as followed: < 200; 200-350; 350-500 and > 500 cells/µL.

85 **2.7 Malaria diagnosis**

86 Two methods were used to diagnose malaria infection namely Giemsa-stained blood films and the
87 ACCUCARE one step Malaria Test® RDT.

88 **2.7.1 Thick blood smears**

89 Thick blood films were performed using the protocol previously described by Cheesbrough (2004) [5].
90 Briefly, thick smears that were air-dried for 30 minutes, was stained with 10% Giemsa for 20 minutes.
91 Thereafter, stained slides were allowed to air dry and stored not more than one day until microscopic
92 examination. Microscopy was used for identification of malaria parasites by a senior. Thick blood films
93 were considered positive when asexual forms (trophozoites and schizonts) and or gametocytes were
94 present in the blood film. Slides were declared negative after observing at least 100 high power fields
95 without detecting any parasites. In order to ensure quality assurance of parasitological data, thick
96 smears-based results were classified as valid (positive or negative slides) and invalid (not read slides)
97 as outlined in literature [9].

98 **2.7.2 ACCUCARE one step Malaria Test®**

99 The ACCUCARE Malaria Pf/Pv test (Labcare Diagnostics, India) is an immunochromatographic test
100 for the qualitative detection of antibodies of all isotopes (IgG, IgM, IgA) specific to *Plasmodium*
101 *falciparum* and *Plasmodium vivax* simultaneously in human serum, plasma or whole blood. It contains
102 a membrane strip, pre-coated with recombinant malaria Pf capture antigen (MSP, CSP) on test band
103 1 region and with recombinant malaria Pv antigen (MSP, CSP) on test band 2 region. The
104 recombinant malaria Pf/Pv antigen (MSP, CSP)-colloid gold conjugate and serum sample moves
105 along the membrane chromatographically to the test regions and forms a visible line as the antigen-
106 antibody-antigen gold particle complex forms [10].

107 The diagnosis of malaria infection using this test was performed according to the manufacturer's
108 instructions. Briefly, 10 µL of serum was added to the sample well "S". Then, 1 drop of the assay
109 chase buffer was added. Results test were read after 10 to 20 minutes [10].

110 **2.8 Ethical approval**

111 This study was carried out in conformance with the guidelines for human experimental models in
112 clinical research as stated by the Cameroon Ministry of Public Health and the Helsinki declaration.
113 Besides, the ethical clearance was sought close to Institutional review board (IRB) of Douala under N°
114 CEI-UD/273/10/2015/T. The aim and objectives of the study were explained to them in the language

they understood best (French or English), and their questions were answered. Only individuals who signed an informed consent form for their participation were enrolled. Participation in the study was strictly voluntary and patients were free to decline answering any question or totally withdraw if they so wished at any time. All malaria positive patients were treated on the spot accordance to the national treatment guidelines.

2.9 Statistical analyses/methods

All data were verified for consistency, coded, and keyed in an Excel sheet. Thereafter, statistical analyses were performed with SPSS 20.0 for Windows (SPSS, Chicago, IL, USA). Data were summarized in table as percentages with 95% confidence interval (95%CI) or mean $\pm$ standard deviation (SD) for qualitative and quantitative variables respectively where appropriate. Student's test was used to compare differences for normally-distributed variables. Chi-square test (χ^2) or Fisher's exact probability were computed to compare categorical variables. Significant levels were measured at 95% CI with significant differences recorded at $P < .05$.

After all the tests were performed the values for sensitivity, specificity, predictive values and accuracy were calculated using light microscopy as a gold standard. The variables measured were the number of true positives (TP), number of true negatives (TN), number of false positives (FP) and number of false negatives (FN). Sensitivity was calculated as $TP/(TP+FN)$, Specificity was calculated as $TN/(TN+FP)$, Positive predictive value (PPV) was calculated as $PPV=TP/TP+FP$, Negative predictive value (NPV) was calculated as $NPV=TN/TN+ FN$, Accuracy = $(TP+TN)/\text{number of all tests}$ and the overall measure of reliability= $(TP \times TN) - (FP \times FN)/(TP+ FN) (TN+FP)$.

The agreement between light microscopy and RDT was appraised by computing the Cohen's Kappa index [11]. Its interpretation was made based on the scale by Landis and Koch (1977) who splitted the kappa index into five categories namely very poor (< 0.00), poor ($0.00-0.20$), moderate ($0.21-0.60$), good ($0.61-0.80$) and excellent (≥ 0.81) [9].

3. RESULTS

3.1 Baseline characteristics of the participants

A total of 723 patients were included in the study. Majority of them were females (74.3%; $P < .0001$). The mean age was 39.49 ± 11.17 years old and this variable ranged between 4 and 74. In addition, patients aged 20-49 years old accounted for more than two thirds of the participants (79.7%, $P < .0001$), followed by more than 49 years (18.5%) and less than 19 years old (1.8%). All patients

studied had a CD4 cells count ranging between 6 and 1369 cells/ μ L. As regards CD4 count, 122 (16.9%) patients had CD4 below 200 cells/ μ L, 192 (26.6%) between 200 and 350 cells/ μ L, 171 (23.7%) between 200 and 350 cells/ μ L and 238 (32.9%) had CD4 > 500 cells/ μ L ($P < .0001$). The mean CD4 count of these patients was 427 cells/ μ L (sd = 257).

3.2 Prevalence of malaria

Overall, *Plasmodia* were found in 121 (16.7%; 95%CI: 14.2%-19.6%) and 91 (12.6%; 95%CI: 10.3%-15.3%) using Giemsa-stained blood films and RDT respectively. Malaria prevalence was higher in females (12.9%) compared to their male counterparts (11.5%). On average, malaria prevalence was increasing with age of participants. Malaria prevalence was 0%, 12.7% and 13.4% in below 19 years old, 19-49 years old and above 49 years old respectively. Furthermore, none statistically significant differences were found.

3.3 Diagnostic performances of ACCUCARE one step Malaria Test[®]

Out of 121 positive tested by the Giemsa-stained light microscopy, 91 have been found to be positive by the RDT while 30 were negative. On the other hand, out of 602 which were negative by Giemsa stained light microscopy, none were positive by the RDT (Table 1). Thus, the sensitivity and specificity of RDT was 75.2% and 100% respectively as summarized in Table 2. Besides, the agreement between both methods was excellent ($\kappa = 0.835$; $P < .0001$). This matter was a good concordance between the methods because of an accuracy of 95.8% (Table 2).

Table 1. ACCUCARE one step Malaria Test[®] results compared to Giemsa stained light microscope

		RDT results		
		Positive	Negative	Total
Giemsa stained light microscopy results	Positive	91 (TP)	30 (FP)	121
	Negative	0 (FN)	602 (TN)	602
	Total	91	632	723

*Data are presented as frequency. RDT = Rapid diagnosis test, TP = True positive, FP = False positive, FN = False negative, TN = True negative

Table 2. Diagnostic performance of ACCUCARE one step Malaria Test® using light microscopy as gold standard

Performance	Values
Sensitivity, % (95%CI)	75.2 (66.8-82.1)
Specificity, % (95%CI)	100% (99.4-100)
Positive predictive value, % (95%CI)	100% (95.9-100)
Negative predictive value, % (95%CI)	95.3 (93.3-96.7)
Accuracy, % (95%CI)	95.8 (94.1-97.1)
Kappa index, (standard error, <i>P</i>)	0.835 (0.029; <i>P</i> < .0001)

**95%CI: Confidence interval; P < .05 are considered significant*

4. discussion

Reliable and convenient diagnosis method is critical to fight against malaria infection. Clinic- and microscopy-based diagnoses are commonly used in malaria endemic areas which are often resources-constrained. Unfortunately, several concerns emerged about their cost-effectiveness to efficiently control malaria due to their intrinsic limitations. Clinic diagnosis is low sensitive and specific mainly in low transmission areas. Microscopy is time-consuming, labor-intensive and depends on quality of reagents and competence of the microscopist. Thus, rapid diagnostic tests (RDTs) may represent an interesting and reliable alternative especially in remote areas where electricity is missing. This study aimed at appraising the performance of a RDT for diagnosis of malaria in people living with HIV infection.

The malaria prevalence was 16.7% in the participants. This value is lower than that found by many reports [12-14]. These authors found 31.76%, 24.0% and 74.3% respectively. Conversely, our value is higher than the 11.75% found by Tay et al. [15] in Ghana. Differences in sample size, study design, study period, study area along with genetic background and behavioral patterns of individuals can explained the discrepancies observed between these study and ours.

A sensitivity of 75.2 % (95%CI: 66.8%-82.1%) in this study. This value is lower than that of Suchita et al. [16] and Khatib et al. [17] who found 95.24% and 92.95% in India. This finding outlines the fact that the RDT was not in line with the gold standard in 25% of patients. Thus, a significant fraction of population would not have received treatment if the test was used alone for malaria diagnosis. As a

result, starting on malaria-related treatment will have been delayed in these patients and consequently increasing the risk of severe malaria.

Specificity of 100% was recorded and therefore no falsely-positive case was reported in this study. This is in line with Suchita et al. (2013) but slightly different from Khatib et al. (2016) who found 99.97% [16-17]. However, these findings witness all the highly specific nature of the ACCUCARE one step Malaria Test[®] to exclude any case of malaria in febrile patient in general population as well as PLWHIV. It is particularly important in the context of reducing drug exposition to patients who do not need antimalarial drugs. Thus, overtreatment of patients could efficiently mitigated and consequently the drug pressure which accounts mainly for appearance and spreading of drug-resistant malaria parasites. This would be helpful to extend the use of artemisinin-based therapeutic regimens used for treating malaria in most of endemic countries [1].

Positive and negative predictive values of this RDT were high (100% and 95.3% respectively). These characteristics are particularly interesting especially in remote malaria endemic areas along with hard-to-reach population where health facilities are not often available. Besides, electricity source is also often missing which is needed to perform microscopy-based malaria diagnosis. Thus, utilization of RDTs, which do not request electricity, of high diagnostic performances would be particularly cost-effective for control malaria in such areas.

5. CONCLUSION

This study was designed to evaluate the performance of a RDT for diagnosis of malaria in people living with HIV infection. The study showed a good performance of the RDT in term of specificity, PPV, NPV, accuracy and agreement. Thus, this test might represent a good alternative to the standard method for diagnosis of malaria in PLVIH along with general population. Further studies are needed to confirm its long term performances.

213

214 **CONSENT**

215 All authors declare that 'written informed consent was obtained from the patient (or other approved
216 parties) for publication of this original research article. A copy of the written consent is available for
217 review by the Editorial office/Chief Editor/Editorial Board members of this journal.

218

219 **ETHICAL APPROVAL**

220 All authors hereby declare that all experiments have been examined and approved by the appropriate
221 ethics committee and have therefore been performed in accordance with the ethical standards laid
222 down in the 1964 Declaration of Helsinki. An ethical clearance was sought close to Institutional
223 Review Board (IRB) of Douala under N° CEI-UD/273/10/2015/T.

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