Performance of apDia Malaria Antigen ELISA for the detection of *Plasmodium spp.* infections in blood donors at the Yaounde Central Hospital, Cameroon

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ABSTRACT

Background: Malaria transmission through blood transfusion can lead to serious and even deadly complications in infected blood recipients if not adequately managed. A cost-effective method for routine screening of blood donors could therefore improve the security of transfused patients. The objective of this study was to evaluate the performance of apDia Malaria Antigen ELISA test for the detection of *Plasmodium spp*. pLDH antigen (pLDH ELISA) in blood donors at the Yaounde Central Hospital Blood Bank.
Methods: A cross-sectional survey on *Plasmodium* infections among 165 blood donors was carried out between August and December 2019. Using EDTA coated tubes, blood samples were collected and analysed for the presence of malaria parasites through pLDH ELISA and pLDH RDT. Using the microscopy as the gold standard, the performance parameters of ELISA pLDH, i.e. sensitivity, specificity, positive and negative likelihood ratios (LR +/-), and predictive values were determined and compared to that of a pLDH RDT.
Results: The overall prevalence of *Plasmodium* infections in tested blood donors was 15.7% (26/165) and 38.8% (63/165) from the pLDH RDT and the pLDH ELISA tests respectively versus 18.8% (31/165) obtained from the microscopy. *Plasmodium falciparum* species was present in 100% of the infected donors. Only one case of mixed infection (*Plasmodium falciparum / Plasmodium malariae*) was recorded. The pLDH ELISA test displayed a sensitivity of 93.3%, a negative predictive value of 97.8%, a positive predictive value of 44.4%, a positive likelihood ratio of 3.3 and a negative likelihood ratio of 0.09. The pLDH RDT had lower performance parameters with a sensitivity of 22.5%, a positive predictive value of 26.9%, a negative predictive value of 82.7%, a positive likelihood ratio of 1.6 and a negative likelihood ratio of 0.9. However, the specificity (71.5%) of the pLDH ELISA test was lower than that of the pLDH RDT kit (85.8%).
Conclusion: The current study provides a strong diagnostic evidence for apDia Malaria Antigen ELISA, making it an interesting tool for mass screening of blood donors to reduce the risk of transfusion malaria in Cameroon.

Keywords : Performance, apDia Malaria Antigen ELISA, pLDH antigen, *Plasmodium spp*., blood donors, Cameroon.

1 BACKGROUND

Malaria remains one of the deadliest infectious diseases in the world, with more than 228 million cases in 2018 and an estimated associated mortality of more than 405.000 deaths [1]. The causative pathogen, *Plasmodium* parasite, is mostly transmitted through the bite of an infected *Anopheles* mosquito. However, transmission can also occur during blood transfusion to patients. Epidemiological studies have shown that the prevalence of asymptomatic *Plasmodium* parasitemia in potential blood donors in malaria-endemic areas ranges from 0.67% to over 55% (with a median prevalence of 10.2%) [2]. A study conducted in Yaounde Cameroon revealed 6.5% prevalence of *Plasmodium* infections in blood donors at the Yaounde Central Hospital Blood Bank [3]. Transfusion-related malaria is particularly common in countries where blood donation has become a commercial transaction and where blood donors come from underprivileged communities.

The risk of *Plasmodium*-infected blood transfusion is exacerbated by the difficulty of identifying infected donors carrying low-level parasitemia (1-2 parasites/µl) [4], as well as the ability of the parasite to survive during the storage of donated blood at 2-4°C for several days to weeks [5]. Indeed, any blood component can harbor *Plasmodium* parasites, the most common cases of transfusion transmission are found in whole blood and red blood cell concentrates [6], and serious complications may threaten the life of the recipients of infected blood if not adequately managed. Thus, the management of transfusion-transmitted malaria cases is a major challenge in malaria-endemic countries such as Cameroon. Therefore, the identification of sensitive, specific, affordable and easily reproducible methods for mass screening of blood donors is of high interest [3]. The reference parasite detection technique (microscopy) that is widely used in the routine diagnosis of malaria amoin the patients in medical consultation, is not adequate for routine use in Blood Banks because it is labor-intensive and time-consuming to examine huge numbers of samples [4]. A number of alternative techniques have been developed for laboratory diagnosis of malaria, that can be used to screen potential blood donors, such as rapid diagnostic tests (RDTs), enzyme-linked immunosorbent assays (ELISA) and molecular biology tests.

However, RDTs have low sensitivity related to low antigen levels, ranging from 100 to 1000 parasites/µl blood [5], and molecular biology tests are very expensive and require high-tech facilities in addition to technical expertise which is scares in endemic countries [5]. Indeed, owing to its affordability and ease to use, ELISA tests for the detection of the pLDH antigen of *Plasmodium spp.* may be a solution for safe malaria transfusion in endemic areas. In addition, the pLDH antigen disappears within 24 hours after effective antimalarial treatment. It is therefore considered an accurate marker for the presence of *Plasmodium* in blood [7]. In Cameroon, although there has been a study to evaluate the performance of the DRG Malaria Antigen ELISA in potential blood donors in the south-western region [5], little is known about the performance parameters of the apDia Malaria Antigen ELISA. Moreover, there are very few reviews data on the performance parameters of pLDH ELISA in blood donors.

Currently in Cameroon, antimalarial drugs are routinely administered to blood recipients as a presumptive treatment. Not only does this treatment entail additional costs for the patient, but the elimination of *Plasmodium* parasites from the blood may become challenging is case of drug resistance. Indeed, malaria transmitted by blood transfusion is a reality in Cameroon, owing to the difficulty to identifying potential infected donors (most being asymptomatic carriers of the parasites). Such a nosocomial infection endangers the life prognosis of the patient. A reliable and affordable screening test that can easily be added to the panoply of tests that already exist in the Blood Banks for transfusion safety may significantly advance the strategies towards the eradication of transfusion transmitted malaria in country.

In order to guide the choice of the method for detection of asymptomatic *Plasmodium* carries among blood donors in Cameroon, we evaluated the performance of the apDia Malaria Antigen ELISA based on the detection of pLDH antigen of *Plasmodium spp*.

2 METHODS

2.1 Study site

The study was conducted at the Blood Bank of the Yaounde Central Hospital (YCH) located in Yaounde, the capital city of Cameroon. Situated in the Central Region of the country, the Yaounde city is split into 07 communes, namely: Yaounde I, Yaounde II, Yaounde III, Yaounde IV, Yaounde V, Yaounde VI and Yaounde VII. In 2019, the overall population of Yaounde was estimated at 4.100.000 inhabitants with 13558.1 inhabitants per km^2 [8]. The climate in this area is sub-equatorial type with a big dry season (December-March) interrupted by rare rains, a small dry season (May-August) with some stormy rains and a big rainy season (August-November) [9]. Although Yaounde is located in the equatorial forest domain, the expansion of settlements has considerably reduced the forest landscape, which is now limited to the surrounding rural areas. The city is 20 km wide and about 25 km long. Owing to the local ecological and climatic features, malaria transmission is permanent throughout the year with seasonal variations [10]. Within the country's health pyramid, the YCH facilities correspond to tertiary level, providing health services to more than 20.000 patients [11]. The YCH Blood Bank unit is the reference Blood Bank for Centre region of Cameroon; blood donors come from Yaounde and surrounding villages. It has an appropriate technical platform (apparatus and equipment necessary for the collection and biological qualification of blood bags) for the type and scope of the current study.

2.2 Study design and framework

A cross-sectional survey on malaria infections among the blood donors visiting the YCH Blood Bank was carried out during a 3-month period from August to October 2019. A flowchart summarizing the process of blood donor selection and blood sampling is provided in fig. 1. The objectives of the study were duly explained to the blood donors in English or in French and the information notice was provided as well. Before enrollment in the study, all the participants were requested to provide a signed informed consent before completing the medical screening from for a blood donation. Then blood was collected in a dry tube for the pre-screening tests. After a favorable opinion from the physician based on the results of the pre-screening tests, a blood bag with 2 collection tubes (EDTA and dry) was taken from the candidate for donation. The blood bag and the EDTA tube were stored at +2-6°C; the dry tube was used for biological qualification tests (HIV, hepatitis B, hepatitis C and syphilis). The blood bags that did not comply with the biologic qualification tests were destroyed and therefore eliminated from this study. The blood contained in EDTA tubes and corresponding to blood bags ready for transfusion was subject to malaria testing using the pLDH ELISA test kit, pLDH RDT and microscopy.

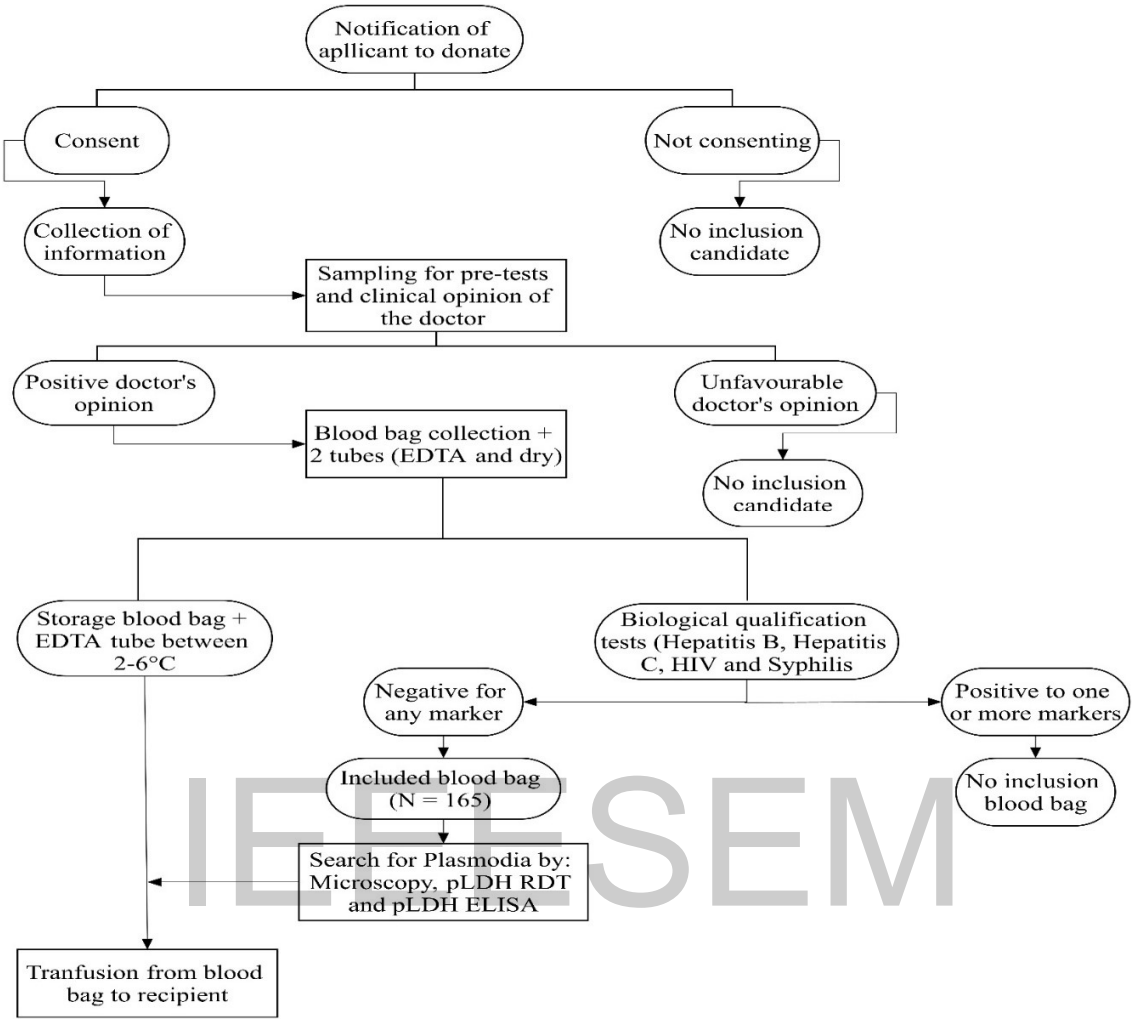

Fig. 1. The flowchart shows the selection of study participants

2.3 Inclusion and exclusion criteria

All the blood bags qualified for donation according to the biological selection criteria and for which the donors have signed the informed consent form were included in the study. Were excluded from the study any blood bag from a donor who consented to participate in the study but not qualified for blood donation, e.g. tested positive for HIV, hepatitis C, hepatitis B and/or syphilis.

2.4 Sampling technique and sample size

The minimal size of study population was calculated with regard to the following formula used for diagnostic studies as defined by Hajian-tilaki *et al*. [12].

$$N = \frac{[Z_{\alpha/2}\sqrt{P_0(1-P_0)} + Z_{\beta}\sqrt{P_1(1-P_1)}]^2}{(P_1 - P_0)^2}$$

Where P_0 denotes the pre-determined value of sensitivity of test apDia Malaria Antigen ELISA (100%); P_1 is the value of sensitivity under alternative hypothesis observe in the field for the test apDia Malaria Antigen ELISA (94%) [7]. The parameters $Z_{\alpha/2}$ and Z_{β} denote the percentiles of standard normal distribution and α, β are the probability of type I and type II errors respectively. In this study $\alpha = 0.05$, $Z_{\alpha/2} = 1.96$, and $Z_{\beta} = 0.84$. The estimated minimal sample size was $N = 11.05$. A total of 165 blood donors were enrolled in this study.

2.5 Laboratory diagnosis of malaria

The apDia Malaria Antigen ELISA kit (apDia bvba, Raadsherenstraat 3, 2300 Turnhout, Belgium) supplied by *Advanced Practical Diagnostics* based in Belgium. This kit is composed of two 96 well microplates, one negative control, two positive control, the lysing buffer, two conjugate solutions (1 and 2), two washing solutions, two chromogen solutions and a stopping solution.
The Rapid Diagnostic Test (RDT) kit CareStart™ Malaria pLDH (PAN) has been supplied by the company Bioecoms Sarl located in Yaounde. This kit contains 25 test cassettes, an assay buffer, pipettes, lancets and alcohol swabs.
Biological analyses of the samples were carried out in the laboratory of the YCH Blood Bank and microscopy in the laboratory of the School of Health Sciences of the Catholic University of Central Africa.

2.6 Diagnosis by ELISA pLDH

The detection of the pLDH antigen was performed by the ELISA Malaria Antigen Sandwich Test which detects by immunocapture based on monoclonal antibodies against pLDH, the pLDH of all *Plasmodium* species. The blood samples are pipetted into the wells to bind to the immobilized antibody. After washing to remove unbound material, pLDH is revealed by addition of a biotinylated anti-pLDH monoclonal antibody which is also pan-specific. After removal of excess biotinylated antibody, streptavidin peroxidase is added. After a final wash, the peroxidase activity is revealed by addition of the substrate solution based on 3,3',5,5' Tetra Methyl Benzidine (TMB) and hydrogen peroxide (H_2O_2). The reaction is then stopped by adding dilute sulphuric acid solution (see detailed description can be found in supplementary file 1).
To detect infected samples, the plates were read by spectrophotometry at 450 nm and the reference wavelength was fixed at 630 nm. The Cutoff (positivity threshold) of each sample was determined by multiplying the mean optical densities of the negative controls by 3. The antigen index (AI) was obtained by dividing the optical density of the samples by the cutoff value. An AI ≤ 0.8 was considered negative, an AI between 0.8 and 1.0 was considered doubtful, and an AI ≥ 1.0 was considered positive.

2.7 Diagnosis by RDT pLDH

For diagnosis using the RDT kit CaraStart™ Malaria PAN (pLDH), the sample wells of the cassettes were filled with about 5 µl of blood from the EDTA container. Three drops of buffer were added to the blood in the sample wells, the mixture was incubated for a few minutes and to allow its flow to the result window on the cassette. After 20 minutes, the cassette was then checked for the appearance of colored bands on the result window.
The test was interpreted as positive if a colored bands appeared at the control region of the cassette and at the test region, while the test was interpreted as negative if only one single colored band appeared at the control region of the cassette and none at the test region.

2.8 Diagnosis by microscopy

A thick drop (TD) and a thin spread were made on slide and stained with May Grunewald Giemsa (MGG) as previously described [13]. Each slide was microscopically read for *Plasmodium* and the parasite density was established on the TD. The identification of the *Plasmodium* species was carried out on the thin spread [14]. The slides were examined by two expert laboratory technicians who were blind to the results of other tests. Each technician carefully examined the thick smear and the blood smear field by field by continuously adjusting the focus for field examination. A slide was declared negative when the technician had carefully examined at least 100 fields. When the species diagnosis was uncertain the technician carefully examined an additional 100 fields to identify a potential mixed infection [14]. For the parasite count, if after counting 200 leukocytes we found 100 or more parasites, the result was recorded as the number of parasites per 200 leukocytes. If, after counting 200 leukocytes, we found 99 parasites, or less, the count was continued up to 500 leukocytes. The parasite density (PD) was expressed as the number of parasites per µl of blood according to the formula.

$$PD = \frac{\text{Number of parasites}}{\text{Number of leukocytes}} \times 8000$$

With 8000 the number of leukocytes per microliter of blood in a normal adult.

2.9 Data analysis

The data on malaria infections collected using the three diagnosis methods were recorded and processed using EpiInfo 7.0 and Excel 2016 software. These data were then analyzed using GraphPad software. The standard curve (Antigen Index versus pLDH concentrations) was generated on semi-logarithmic graph paper from the recombinant antigen (rpLDH) provided in the apDia Malaria Antigen ELISA kit as a positive control. The study by Atchade *et al.* [7], revealed that 0.08 ng/ml pLDH corresponds to 1 parasite/µl and from this result we plotted on an Excel 2016 spreadsheet a second standard curve giving the pLDH concentration versus the number of parasite/µL. This curve was used to estimate the parasite density of each sample in the pLDH ELISA. The prevalence of malaria was calculated and expressed as a percentage by

dividing the number of donors infected with *Plasmodium* parasites by the total number of donors examined [4]. Among the 10 performance indicators of a diagnostic test defined by Bolboaca *et al.* [15], we selected 6 variables that are the most relevant to determine the performance parameters of the tests evaluated (pLDH ELISA and pLDH RDT) namely: Sensitivity = [true positive / (true positive + false negative) x 100]; specificity = [true negative / (true negative + false positive) x 100]; positive predictive value = [true positive / (true positive + false positive) x 100]; negative predictive value = [true negative / (true negative + false negative) x 100]; positive likelihood ratio = [sensitivity / 1-specificity] and the negative likelihood ratio = [1-sensitivity / specificity]. Microscopy was used as the gold standard [16]. Doubtful results were not taken into account in the calculations. The chi-square test was used to compare the performance of the pLDH ELISA kit to that of the pLDH RDT, using the microscopy gold standard. The confidence interval was set at 95% and the statistical significance at a *P-value* < 0.05.

3 RESULTS

3.1 Socio demographic characteristic, infection rates and seniority in blood donation

The socio-demographic data and the infection rates of the examined blood donors are presented in table 1. Of the one hundred and sixty-five (165) blood donors enrolled in the study, the majority were men accounting for 89.7% (146/165) versus 10.3% women (17/165).

Table 1. General characteristics of donors

Parameters	Frequencies (%)	Number of parasitized donors by microscopy (n=31)	Positivity rate (%)	Chi-square	*P-value*
Age (years)					
18 - 24	41 (24.9)	8	19.5	0.148	0.28
25 - 65	124 (75.1)	23	18.5		
Gender					
Male	148 (89.7)	28	18.9	0.016	0.89
Female	17 (10.3)	3	17.6		
Donors aging					
Regular donors	66 (40.0)	10	15.2	0.215	0.70
New donors	99 (60.0)	21	21.2		
Profession					
Student	50 (30.3)	10	20.0	74.596	0.09
Shopkeepers	26 (15.7)	5	19.2		
Unemployed	22 (13.3)	4	18.2		
Technician	13 (7.9)	1	7.7		
Official	10 (6.0)	1	10.0		
Contractor	7 (4.2)	1	14.3		
Driver	7 (4.2)	2	28.6		
Security agent	7 (4.2)	3	42.8		
Teacher	6 (3.6)	1	16.7		
Engineer	10 (6.1)	1	10.0		
Mason	3 (1.8)	1	33.3		
Welter	4 (2.4)	1	25.0		
Borough					
Bastos	3 (1.8)	0	0.0	73.716	0.35
Biyemassi	21 (12.7)	6	28.6		
Briqueterie	9 (5.4)	1	11.1		
Cite verte	4 (2.4)	1	25.0		
Damas	9 (5.4)	2	22.2		
Ekounou	12 (7.3)	4	33.3		
Emana	6 (3.6)	1	16.6		
Essos	14 (8.5)	2	14.3		
Etoukebe	8 (4.8)	2	25.0		
Melen	12 (7.3)	2	16.6		
Mendong	11 (6.7)	1	9.1		
Mimboman	16 (9.7)	4	25.0		
Nkoabang	10 (6.1)	0	0.0		
Nkolbisson	7 (4.2)	1	14.3		
Nkolmesseng	7 (4.2)	0	0.0		
Olezoa	12 (7.3)	4	33.3		
Soa	4 (4.2)	0	0.0		

The mean age of the participents was 30 (±8) years. Sixty percent (60%) of them were novice donors. Among all donors, 12 occupations were identified. Students were represented at 30% (CI: 23.0 – 36.9%); followed by shopkeepers (15.7%, CI: 7.8 – 18.1%) and the unemployed (13.3%, CI: 5.4 – 14.5%). Other occupations such as teacher, official, driver, technician, contractor, security agent, engineer, mason and welter were weakly represented with percentages below 13%. Regarding the residence of the donors, around 30% of the blood donors came from populated quarters including Biyemassi (12.7%, CI: 7.6 – 17.8%), Mimboman (9.7%, CI: 5.2 – 14.2%), Essos (8.5%, CI: 4.2 – 12.7%). The other districts were under represented ranging from 1.8% (CI: 0.0 – 3.8%) in Bastos to 7.3% (CI: 3.3 – 11.3%) in Ekounou. No significant difference was recorded between the infection rates between men and women ($X^2 = 0.016$; df = 2; p = 0.89), age groups ($X^2 = 0.148$; df = 2; p = 0.28), or seniority in blood donation ($X^2 = 0.215$; df = 2; p = 0.70).

3.2 Infection detection rates and associated parasitemia by diagnostic method

Microscopy and pLDH ELISA were used to determine the parasite density and not the pLDH RDT because this method does not allow a quantitative analysis of parasitemia. Infection rates and parasite densities as obtained from different diagnostic methods are given in fig. 2 and fig. 3 respectively; the distribution of *Plasmodium* species is given in table 2. The detection of *Plasmodium* positive blood donors was significantly higher with pLDH ELISA (38.2%), compared with microscopy and pLDH RDT (16 – 19%) ($X^2 = 41.895$; df = 2; p = 0.00). All the donors were infected with *P. falciparum*; only one mixed infection (*P. falciparum* / *P. malariae*) was identified. The infection from microscopic analysis was significantly higher in October (26.1%) compared with August and September ($X^2 = 330.00$; df = 4; p = 0.00). Parasite density in microscopy positive samples ranged from 16 to 4080 parasites/µl blood with a mean of 280 ± 138.1 parasites/µl blood. In contrast, parasite density in pLDH ELISA positive samples ranged from 1 to 52 parasites/µl blood with a mean of 12 ± 4.9 parasites/µl.

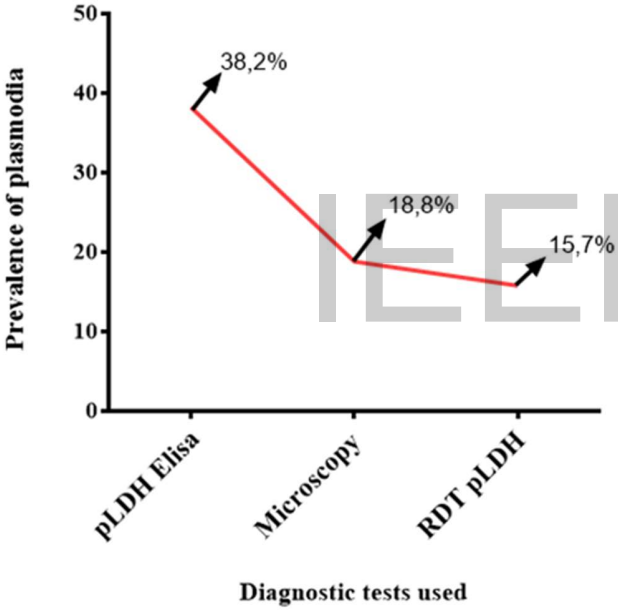

Fig. 2. Prevalence of plasmodia by diagnostic methods

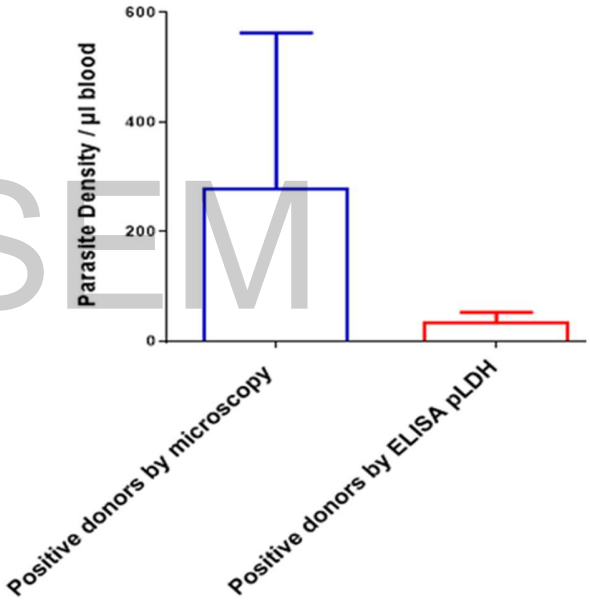

Fig. 3. Parasite density in microscopy and pLDH ELISA positive subjects

3.3 Performance of the apDia Malaria Antigen ELISA

Data on the test performance are provided in table 3. Using the microscopy gold standard, the sensitivity of pLDH ELISA was higher (93.3%, CI: 77.9 – 99.2%) than that of pLDH RDT (22.6%, CI: 9.6 – 41.1%) ($X^2 = 8.449$; df = 2; p = 0.00). Conversely, the specificity of pLDH RDT was significantly higher (85.8%, CI: 78.7 – 91.2%) versus pLDH ELISA (71.5%, CI: 62.7 – 79.3%) ($X^2 = 0.856$; df = 1; p = 0.35). The Positive Predictive Value (PPV) (44.4%, CI: 37.3 – 51.8%) and Negative Predictive Value (NPV) (97.8%, CI: 91.9 – 99.4%) were higher with the pLDH ELISA compared with pLDH RDT [(PPV: 26.9%, CI: 14.5 – 44.4%; NPV: 82.7%, CI: 79.6 – 85.4%)]. The Likelihood Ratio of a positive result (LR+) (3.3, CI: 2.4 – 4.4) and the Likelihood Ratio of a negative result (LR-) (0.09, CI: 0.1 – 0.4) were higher with the pLDH ELISA compared with pLDH ELISA compared with pLDH RDT [(LR+: 1.6, CI: 0.7 – 3.4; LR-: 0.9, CI: 0.7 – 1.1)].

Table 2. Distribution of *Plasmodium* species

Collection period	Numbers of donors tested (N=165)	Numbers of donors parasitized by microscopy (n=31)	Positivity rate (%)	*Plasmodium* species (n=31)
August 2019	50	8	16.0	*P. falciparum* (8/8) *Mixed infection (1/8)
September 2019	69	11	15.9	*P. falciparum* (11/11)
October 2019	46	12	26.1	*P. falciparum* (12/12)

*Mixed infection : *P. falciparum* / *P. malariae*

4 DISCUSSION

The current study revealed considerable rates of infections among men blood donors as well as women donors (18 – 19%) of the YCH Blood Bank. Although this data is consistent with previous studies in Nigeria and Benin [7, 17], the sample size of the women blood donors at the YCH Blood Bank was very low (n=17) compared with men (n=148). This small sample size is likely to dissimilate their susceptibility to malaria infection, since women were found more parasitized then men blood donors in Ghana [18]. Also, there was no significant difference in the age groups of parasitized blood donors of the YCH. This may be due to the fact that the majority of participants were adults (25 – 65 years old), therefore they might have already acquired immunity to malaria as reported in previous studies conducted in Cameroon [5]. A high rate of infection was recorded in new donors (21%) compared with regular donors (15%), although the difference was not significant. This finding support the widespread evidence in the blood transfusion literature that first time donors are high-risk group [19] because they have ambiguous reasons for donating [20], e.g. access to specific biological tests (e.g. HIV), financial compensation, etc. Indeed, the majority of the donors who participated in this study belonged to social groups with limited income, such as students, small traders and the unemployed.

In these groups, malaria prevalence as recorded by microscopy was 2 to 3 times higher than those reported in donors from Buea in 2016 (8.1%) [5] and Yaounde in 2013 (6.5%) [3]. This prevalence was rather comparable to those reported by Bassandja in 2013 among voluntary blood donors in Kisangani in the Democratic Republic of Congo (28.3%) by microscopy [21]. Indeed, this finding highly suggest the rise of transfusion malaria in Cameroun, with the *P. falciparum* species, recognized as an agent of serious complications. This upsurge could be explained by a low use of malaria prevention tools and a lack of awareness among at risk donors. On the other hand, the increase in malaria prevalence could also be linked to the level of endemicity in the living areas of the blood donors as well as the seasonality of the study period. Although malaria transmission occurs continuously in the city of Yaounde, the intensity of the transmission is variable according to seasons and the places [22]. The current survey was conducted between August and October 2019, i.e. during the rainy season, which also corresponds to a period conducive to high *Anopheles* densities. According to the study conducted by Doumbe-belisse [22], a moderate to high risk of malaria transmission was recorded in twelve districts of Yaounde (Essos, Ekie, Mvog-Ada, Tongolo, Tsinga, Biyemassi Ecole, Biyemassi lac, Ekounou palais, Nkolbikok, Nkolbisson, Nouvelle route Nkolbisson and Olezoa), including those where the study participants came from. In such counties, the rapid and unplanned urbanization has led to the creation of mosquito breeding sites through anthropic activities, such as car washing and urban agriculture. There, *Plasmodium* parasite transmission is mainly carried out by *Anopheles gambiae* and *An. coluzzii*, two species of the *Anopheles* complex which are the most efficient malaria vectors in Africa [23].

Regarding the performance of diagnosis methods, the sensitivity (93.3%) and the NPV (97.8%, CI: 91.9 – 99.4%) obtained with the apDia Malaria Antigen ELISA recorded in this study were similar to that obtained by Atchade *et al.* in blood donors from Benin, i.e. 94% sensitivity and 99.5% NPV. However, the specificity (71.5%) and the PPV (44.4%, CI: 37.3 – 51.8%) were lower than that reported by Atchade *et al*. (97.5% specificity and 94.3% PPV). These discrepancies could be due to the difference in gold standard technique. Indeed, we used microscopy as the gold standard according to World Health Organization (WHO) guidelines; whereas Atchade *et al.* used Polymerase Chain Reaction (PCR) as the reference test [24]. Nevertheless, the microscopy examination was not efficient enough for screening blood donors carrying low parasitemia as previously reported [25]. Indeed, the level of parasitemia detected by pLDH ELISA (12 ± 4.9 parasites/µl blood) was significantly lower than that of the microscopy (280 ± 138.1 parasites/µl blood). A part from the influence of the gold standard method, the difference in PPV can also be explained by the variability of malaria prevalence in the study areas.

According to Bolboaca [15], a diagnostic test with a positive likelihood ratio between 2 – 5 reflects a moderate diagnostic evidence and that with a negative likelihood ratio of less than 0.1 has a strong diagnostic evidence. The best diagnostic test is one with a positive likelihood ratio greater than 10 and a negative likelihood ratio less than 0.1.

Table 3. Diagnostic performance of apDia Malaria Antigen ELISA and CareStart™ Malaria PAN (pLDH) RDT using microscopy as reference method

Diagnostic tests	Microscopy	
	Positive (n=31)	Negative (n=134)
apDia Malaria Antigen ELISA		
Positive	28	35
Negative	2	88
Doubtful	1	11
CareStart™ Malaria PAN (pLDH) RDT		
Positive	7	19
Negative	24	115
Measures of diagnostic performance	**Value pLDH ELISA**	**Value pLDH RDT**
Sensitivity (95% IC) (%)	93.3 (77.9 - 99.2)	22.6 (9.6 - 41.1)
Specificity (95% IC) (%)	71.5 (62.7 - 79.3)	85.8 (78.7 - 91.2)
Positive predictive value (95% IC) (%)	44.4 (37.3 - 51.8)	26.9 (14.5 - 44.4)
Negative predictive value (95% IC) (%)	97.8 (91.9 - 99.4)	82.7 (79.7 - 85.4)
Likelihood ratio of a positive test	3.3	1.6
Likelihood ratio of a negative test	0.09	0.90

The positive likelihood ratio of 3.3 and a negative likelihood ratio of 0.09 obtained with apDia Malaria Antigen ELISA applied to blood donors of the YCH Blood Bank showed strong diagnostic evidence for apDia Malaria Antigen ELISA with a negative likelihood ratio less than 0.1.

The performance parameters of the apDia Malaria Antigen ELISA kit were compared with an RDT CareStart™ Malaria PAN pLDH. The apDia Malaria Antigen ELISA showed a significantly higher sensitivity (93.3%) than the CareStart™ Malaria PAN pLDH which was 22.6%. According to Bashir *et al*. [26], the higher sensitivity observed in the pLDH ELISA compared to pLDH-based RDTs could be associated with the fact that the ELISA uses 50 µl of blood, whereas the RDTs use only 5 µl of blood. However, a study by Jang *et al.* [25] revealed a high sensitivity of the SD BIOLINE Malaria Antigen PAN pLDH RDT (86.8%). This high sensitivity could be attributed to the target population in which the test was evaluated. Indeed, in the study by Jang *et al*. these were clinically ill patients, so the probability of finding a high density of *Plasmodium* was higher.

5 Conclusion

The prevalence of malaria parasites among blood donors at the YCH is considerably high and the lack of systematic screening of donations puts beneficiaries, particularly immunocompromised patients, children under 5 years old, pregnant women, expatriates and tourists from countries where malaria is not endemic at risk. Based on its performance, the apDia Malaria Antigen ELISA present strong diagnostic evidence making it an interesting tool for mass screening of blood donors in Yaounde. Considering blood scarcity of blood donors in Yaounde and the increasing need for blood transfusion, *Plasmodium* positive donors should be provided with preventive measure and adequate treatment so that blood donation is *Plasmodium* free.

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ADDITIONAL FILE
Additional file 1: Detail description of the protocol for apDia Malaria Antigen ELISA.

AUTHORS' CONTRIBUTIONS
LDA conceived and designed the study protocol, participated in data collection, analysis and interpretation, conducted the research and literature review and co-authored the document. **NSA** participated in the data collection, participated in the analyses and interpretation, and co-authored the document. **MP** participated in the study design, participated in the interpretation of results and critically reviewed the document. **JE** designed and coordinated the study, participated in the statistical analyses and critically reviewed the document. All authors read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS
All the data supporting our findings have been presented in the paper.

ETHICAL CONSIDERATION
Ethical approval (n° 2019/0988/CEIRSH/ESS/MIM) has been obtained from the Institutional Ethics Committee of the School of Health Sciences of the Catholic University of Central Africa (CEIRSH). Written informed consent was obtained from each study participant. Authorization to conduct the study was obtained from the Director of the Yaounde Central Hospital.

CONSENT FOR PUBLICATION
Not applicable.

COMPETING INTERESTS
The authors declare that they have no competing interests.

ABBREVIATIONS
AI: Antigen Index; APDIA: Advanced Practical Diagnostic; CI: Confidence Interval; ELISA: Enzyme Linked Immunosorbent Assay; LR+: Likelihood Ratio of a positive result; LR-: Likelihood Ratio of a negative result; NPV: Negative Predictive Value; PD: Parasite Density; pLDH: *Plasmodium* Lactate Dehydrogenase; PPV: Positive Predictive Value; RDT: Rapid Diagnostic test; TD: Thick Drop; YCH: Yaounde Central Hospital.

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