Performance of PfHRP2 and PfPLDH Rapid Diagnostics Test for Diagnosis of Plasmodium falciparum in Assosa Zone, Northwest Ethiopia

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Abstract

Background

Malaria is a life-threatening infectious disease particularly due to *Plasmodium falciparum (P.falciparum). Plasmodium falciparum* Histidine-Rich Protein 2 (PfHRP-2) and *Plasmodium falciparum* specific Lactate Dehydrogenase (PfPLDH) based rapid diagnostic test are commonly used for malaria diagnosis in malaria endemic countries where microscopic examination is scarce. However, there is limited information on the performance of malaria RDT in rural and semi urban area of Assosa zone, Northwest Ethiopia. Thus, the aim of this study is to determine the performance of PfHRP2 and PfPLDH RDT for diagnosis of *falciparum* malaria against microscopy as reference method.

Methods

Health-facility based cross-sectional study design was conducted in Assosa zone, Northwest Ethiopia from November to December 2018. A total of four hundred and six malaria-suspected participants attending Bambasi, Sherkole, Kurmuk and Assosa health-centers were tested. Finger-prick blood samples were collected for microscopy blood film preparation, RDTs and molecular diagnosis. Statistical analyses were performed using SPSS version 20.

Results

Of the total study participants, 26.4% (107/406) were microscope confirmed *P. falciparum* positive. Using PfHRP2 and PfPLDH RDT, 30.3% (123/406) and 24.1% (98/406) were positive for *P. falciparum*, respectively. The sensitivity of PfHRP2 and PfPLDH was 96% and 89%, respectively, against microscope. The corresponding specificity rates of PfHRP2 and PfPLDH were 93% and 99%, respectively. Similarly, positive predictive value (PPV) and negative predictive value of PfHRP2 and PfPLDH RDT were 84%, 97%, 99% and 96%, respectively. There was an agreement between RDTs (PfPLDH and PfHRP2) and reference microscopy method with a kappa value of 0.86 and 0.90, respectively. Compared to qPCR, the specificity of PfHRP2 (93%) and PfPLDH RDT (98%) were high, though the respective sensitivity of PfHRP2(77%) and PfPLDH RDT(70%) were low.

Conclusion

PfHRP2 and PfPLDH showed reasonable agreement in detecting *P. falciparum* infections. Hence, currently used national malaria RDT kit can be continue to be used in certain malaria endemic areas in Ethiopia. However, Continuous monitoring the performance of PfHRP-2 RDT associated with false negative results is important to consider an alternative malaria RDT like PfPLDH RDT to support control and elimination of malaria in Ethiopia

Backgrounds

Malaria is a life-threatening infectious disease that remains to be a major public health problem

in several developing countries [1]. Among the five major human malaria parasites, *Plasmodium falciparum (60%)* and *Plasmodium vivax* (40%) are the most prevalent in Ethiopia [2]. In line this, *P. falciparum* is the main cause of complicated malaria. The first strategic pillar to reduce morbidity and mortality of malaria is the provision of universal access to malaria prevention, diagnosis and treatment in malaria-endemic countries[3].

Diagnosis of malaria in a clinical setting is usually performed by a combination of clinical and laboratory diagnosis to improve the quality of patients care [4]. However, clinical diagnosis of malaria based on symptoms is not sufficient since it difficult to differentiate malaria-induced febrile illness from that of non-malaria induced febrile illness[5]. As the results, presumptive treatment may lead to antimalaria drug resistant and complication of malaria diseases[6]. Accordingly, the World Health Organization (WHO) recommends that all persons suspected of malaria should receive laboratory diagnosis before the use of antimalaria drug therapy to improve case management and malaria control [7].

In clinical settings, microscopic blood film examination is still the reference test and a routinely used laboratory diagnostic method to guide the clinical case management of malaria at Health center Level. At the community level, malaria Rapid Diagnostic Test (RDT) is commonly used in malaria case investigations, case management, and surveillance in rural and semi urban malaria-endemic areas in sub-Saharan Africa including Ethiopia where microscopic examination cannot be performed [4, 8].The most commonly used RDTs antigen for diagnosis of malaria are Histidine-Rich Protein 2 (HRP2) and parasite-specific Lactate Dehydrogenase (PLDH). *Plasmodium falciparum* Histidine-Rich Protein 2 (PfHRP-2) RDT is specific for diagnosis of *P. falciparum* whereas *Plasmodium falciparum* specific Lactate Dehydrogenase (PfPLDH) and *Plasmodium vivax* specific Lactate Dehydrogenase (PVPLDH) based RDT are specific for diagnosis of *P.falciparum* and *P,vivax,* respectively . On the other hand, Pan-specific PLDH based RDT detect the presence of all human malaria parasites species *(P. falciparum, P.vivax, Plasmodium malariae* and *Plasmodium ovale)* [9, 10].

Studies on the performance of malaria RDT have been well documented in different malaria endemic countries[11-17]. There are several factors that affect the performance of malaria RDT. These includes technical factors such as improper storage and packaging, poor product design, and operator error [18]. Furthermore, low level of parasitemia could result in false negative detection by the PfPLDH RDT, whereas the persistence of PfHRP2 antigen in blood for more than three weeks after antimalaria treatment could result in false positive detection by the PFHRP2 RDT. Recently, false negative cases of PfHRP2 based RDTs have been reported [19, 20]. Such a discrepancy is likely associated with genetic variation and gene deletion of *Pfhrp2* in *P. falciparum* observed in East African countries such as Sudan, Eritrea, and Kenya[21-23].

Therefore, the performance of malaria RDT need to be monitored continuously in different malaria endemic setting and localities to determine its sensitivity and specificity. In line this, global effort of malaria control and elimination will be also supported if quality-assured malaria rapid diagnostic tests are routinely used in malaria endemic countries [3]. To date, there is limited information on the

performance of malaria RDT in rural and semi urban areas of Assosa zone, Northwest Ethiopia. Thus, the aim of this study was to determine the performance of PfHRP2 and PfPLDH rapid diagnostic test for diagnosis of *P. falciparum* malaria against microscopy as reference method.

Methods

Study design and area

A cross-sectional health facilities based study was conducted in Assosa Zone, Northwest Ethiopia from November to December 2018. Assosa Zone is one of the four Zonal administration in Benishangul-Gumuz Regional State and is located 687 km west of Addis Ababa, Ethiopia. Assosa Zone consists of eight woredas with majorities of the woreda is with high malaria transmission intensity based on annual parasite incidence conducted according to 2016/17 report of Health Information Management System from Benishangul-Gumuz regional state.

Study participants

The study participants were all self-presenting clinically suspected malaria patients, whose age were greater than or equal to 5 years, who were attending at four randomly selected health facilities: Assosa Health Centre, Bambasi Health Centre, Kurmuk Health Centre and Sherkole Health Centre. Individual with a history of antimalarial chemotherapy in the last month with severe illness were excluded.

Sample size determination and sampling procedure

Sample size calculations were performed based on single population proportion formula $n = z^2p(1-p)/d^2$[24]; where n = the sample size, z = 1.96 at 95% confidence interval (CI), d = margin of error, p = expected malaria prevalence rate in the locality which is 40% based on the assumption of a microscopy-confirmed prevalence of malaria among symptomatic patients according to 2015 the study[25], d = margin of error at 5% (standard value of 0.05). Subsequently, a total of 406 study participants were calculated and enrolled including 10% non-response rate in this study.

Four health facilities, Assosa Health Centre, Bambasi Health Centre, kurmuk Health Centre and Sherkole Health Centre were selected using simple random sampling technique among eight Health centers in the Assosa Zone. Then, allocation of the study participants to each selected health center was performed based on proportion of confirmed malaria case in each selected woreda, based on annual parasite incidence in 2016/17 report of Health Information Management System from Benishangul-Gumuz regional state [2].

Demographic data and collection of blood samples

Demographic data were collected using an interview-based structured questionnaire. Finger-prick blood sample was collected for direct preparation of thin and thick smear microscopy, Pf/PV based RDT(HRP-

2/PLDH), Pf-based RDT(HRP-2/PLDH) and Dried blood spots (DBSs) on Whatman filter paper for molecular Assay. Methodological workflow is described in Fig 1.

Microscopic blood film examination

Both thick and thin blood smear were prepared on the same slide for each study participant. The thick film was performed to detect and measure the density of the parasite. Asexual parasite densities were determined against 200 white blood cells (WBC), assuming mean WBC count was 8,000/μL, as per the WHO recommendations[26]. The thin film was performed for species identification of malaria parasites. The thick and thin blood smear were stained with 10% buffer-diluted Giemsa stain working solution for 10min and examined by 100x oil immersion objective microscope. Standard operating procedures were used for specimen collection, processing and testing for maintaining a good quality data Each blood film was read by two independent medical laboratory technicians and there was no discrepancies in the result.

Malaria rapid diagnostic tests (RDTs)

The CareStart^{TM} malaria RDTs (Pf/PV (HRP2/PLDH) Ag Combo RDT with Lot code MV 18C64, and Pf (HRP2/PLDH) Ag Combo RDT with Lot code MS 18H61) from Access Bio Ethiopia were used to evaluate their performance against microscopy as reference test. PLDH is specific for *P.vivax* and *P.falciprum* in MV18C64 and MS18H61 Lot, respectively. Briefly, malaria RDTs utilize immunochromatographic methods to detect parasite-specific antigens produced by malaria parasites. This method is lateral flow devices that uses colored antibody that binds to lysed parasite antigen and was carried by capillary action on a nitrocellulose strip and arrested by a capture antibody, resulting in a colored band on a test strip[27] . Each test was interpreted based on the manufacturer's instructions in the package insert.

Molecular Assay

SYBR Green quantitative polymerase chain reaction (qPCR) assay was performed to correct discordant results such as false-positive and negative RDT results, and submicroscopic clinical samples. False positive RDT result is malaria suspected cases with a microscope confirmed negative result. False negative RDT result is malaria suspected cases with a microscope confirmed positive result.

Malaria parasite DNA was extracted from dried blood spots (DBS) using Chelex-Saponin method as describe previously [28]. SYBR Green quantitative PCR (qPCR) Assay were performed to amplify 18S rRNA gene for confirmation of *P.falciparum* using of a pair of forward and reverse primers sequence(*P.falciparum*-specific primers) :FAL-18S-F:AGTCATCTTTCGAGGTGACTTTTAGATTGCT and PLASMO-R2:GCCGCAAGCTCCACGCCTGGTGGTGC[29, 30]. For the quality control, DNA from *P. falciparum* isolates 7G8 (MRA-926) and HB3 (MRA-155) were used as positive controls, water and uninfected samples were used as a negative control in all amplifications . Briefly, qPCR amplification were carried out in the total reaction volume of 20μl containing 7μl Nuclease free water, 10μl of SYBR GREEN master mix, 0.5μl each of the forward and reverse primer, and 2 μl of extracted DNA under the

following PCR cycling conditions: initial denaturation at 95°C for 3minutes, followed by 45 cycles amplification at 94°C for 30 seconds, 55°C for 30 seconds, and 68°C for 1minutes each.

Data quality assurance

Data collectors were trained, and close supervision was conducted during the data collection period. Standard operating procedures were used for specimen collection, processing and testing for maintaining a good quality data. Ten percent of samples were also randomly selected and rechecked blindly to ensure quality control

Data Analysis

The data were entered, cleaned and analyzed using Statistical Package for Social Sciences (SPSS) version 20. The diagnostic performance of malaria RDT against microscopic blood film and qPCR were assessed by calculating sensitivity, specificity and predictive values. The percentages of positive and negative infections were recorded and compared among these diagnostic methods. The agreement between these malaria diagnostic methods were assessed using with a kappa value.

Results

Study participants

Among a total of 406 study participants enrolled in the current study, 57.9% (235/406) were females and the remaining were males. The mean age of the study participant was 24.1 years. The majority of the participants (39.2%) had microscopy parasite density level ranged from 2001-10,000 parasite/μL. Age groups from 15–24 and 25–34 years had the highest parasite density as indicated in Table 1. Parasitemia were higher in male than in females, however, there was no statistically significant association of both gender ($P < 0.339$) and age group ($P < 0.780$) with microscopy parasite density.

Table 1
Distribution of parasite density by sex and age group at Assosa Zone, Northwest Ethiopia, November to December, 2018

Parasite density /µL	Study Participants					
	Sex		Age group in years			
	M No (%)	F No (%)	5–14 No (%)	15–24 No (%)	25–34 No (%)	>34 No (%)
50–200	7(6.5)	5(4.7)	4(3.7)	7(6.5)	0(0.0)	1(0.9)
201–500	4(3.7)	9(8.4)	1(0.9)	5(4.7)	4(3.7)	3(2.8)
501–2000	8(7.5)	8(7.5)	3(2.8)	7(6.5)	4(3.7)	2(1.9)
2001–10000	24(22.4)	18(16.8)	9(8.4)	17(15.9)	10(9.3)	6(5.6)
>10000	9(8.4)	15(14.0)	7(6.5)	10(9.3)	4 (3.7)	3(2.8)
	P < 0.339		P < 0.780			

Microscopy, RDT and qPCR results

From a total of 406 malaria suspected self-presenting febrile patients, 26.4% (107/406) were P. falciparum positive by microscope and the positivity rate was 30.3% and 24.1% (98/406) as determined by PfHRP2 and PfPLDH-based RDTs, respectively (Table 2). Higher numbers of P.falciparum, 33.5% (136/405), were detected by qPCR in comparison with microscopy and malaria RDT. Even though the age group 15–24 years showed the highest infection rate based on all methods, there was no statistically significant association between microscopy (P < 0.825), PfHRP2 RDT (P < 0.981), PfPLDH RDT (P < 0.679) and qPCR (P < 426) with age group in this study.

Table 2

P. falciparum positivity rate using different laboratory methods by age groups at Assosa Zone, Northwest Ethiopia, November to December, 2018

Age group in years	No Examined	Microscopy (N = 406)	Malaria RDT		qPCR (N = 405)
			PfHRP2 (N = 406)	PfPLDH (N = 406)	
		No. positive, (%)	No. positive (%)	No. positive (%)	Positive No (%)
5–14	90	24(5.9)	28(6.9)	22(5.4)	26(6.4)
15–24	161	46(11.3)	50(12.3)	43(10.6)	61(15.1)
25–34	93	22(5.4)	27(6.7)	21(5.2)	28(6.9)
> 34	62	15(3.7)	18(4.4)	12(3.0)	21(5.2)
Total	406	107(26.4)	123(30.3)	98(24.1)	136(33.6)
		P < 0.825	P < 0.981	P < 0.679	P < 0.426

NB: N = Total number examined, No = Total number

Sensitivity and specificity of malaria RDTs

Taking microscope as a reference test, the sensitivity of PfHRP2 and PfPLDH RDTs was 96% (95% confidence interval (CI), 91–99%) and 89%(95% CI, 81–93%), respectively (Table 3). The corresponding specificity rates were 93% (95% CI, 90 to 96%) and 99% (95% (CI, 97 to 100%), respectively. Moreover, the positive predictive value (PPV) and negative predictive value of PfHRP2 and PfPLDH RDT are presented in Table 3. There was a good agreement between rapid diagnostic test (PfHRP2 and PfPLDH) and reference microscopy method with a kappa value of 0.86 and 0.90, respectively.

In comparison with qPCR, lower sensitivity in both PfHRP2 77% (95% CI, 70 to 83%) and PfPLDH RDT 70% (95% CI, 62 to 77%) were detected. In contrast, high specificity of both PfHRP2 93% (95% CI, 89 to 95%) and PfPLDH 98% (95% CI, 91 to 99%) against qPCR were revealed in the study (Table 4). PfHRP2 RDT and qPCR were shown a good measure of agreement with a Kappa value of 0.80.

Table 3

Diagnostic performance of PfHRP2 and PfPLDH RDT as compared to microscope, Assosa Zone, Northwest Ethiopia, November to December 2018

Test		Microscopy		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	% NPV (95% CI)	Kappa (95% CI)
		Pos	Neg					
PfHRP2 RDT	Pos	103	20	96(91, 99)	93(90, 96)	84(76, 89)	99(96, 100)	0.86(0.80,91)
	Neg	4	279					
PfPLDH RDT	Pos	95	3	89 (81,93)	99(97, 100)	97 (91, 99)	96(93, 98)	0.90(.85.0.95)
	Neg	12	269					

Note:Pos = Positive, Neg = Negative, CI = Confidence interval, PPV = Positive Predictive value, NPV = Negative Predictive value

The sensitivity of Pf-PLDH and PfHRP2 antigen band were improved with increasing the level of parasitemia (Table 4). However, four samples were missed by PfPLDH at parasite density of 50–200 /µl with 67% of sensitivity whereas two samples were missed by PfHRP2 at parasitemia of 50–200 parasite/µl with 83% of sensitivity.

Table 4

Sensitivity of PfHRP2 and PfPLDH RDT by level of P.falciparum parasitemia at Assosa Zone, Northwest Ethiopia, November to December, 2018

	Malaria RDT				
	Microscopy	PfHRP2		PfPLDH	
Microscopy Parasite density /µL	Positive No	Positive No	Sensitivity %	Positive No (%)	Sensitivity (%)
50–200	12	10	83	8	67
201–500	13	12	92	10	80
501–2000	16	16	100	13	81
2001–10000	42	41	97	40	98
>10000	24	24	100	24	100

Note: µl = microliter, No = Total number

False positive and negative RDTs

Higher numbers of positive PfHRP2 antigen bands, PfHRP2 false positive rate of 6.7% (20/299), were occurred in the absence of PfPLDH antigen band. The number of samples missed by PfPLDH, PfPLDH

false negative rate of 11.2% (12/107), were three times higher than PfHRP2 RDT (Table 3). In contrast to the reference microscopy, higher false negative rate in both PfHRP2 RDT23.5% (32/136) and PfPLDH RDT30.1(41/136) were occurred in comparison with qPCR. On the other hand, lower false positive rate were shown in both PfPLDH 1.1% (3/269) and PfHRP2 7.1% (19/269) in comparison with qPCR for the diagnosis of malaria (Table 5).

Table 5
Performance of Microscopy, PfHRP2 RDT and PfPLDH RDT in comparison with qPCR for the diagnosis of P. falciparumat Assosa Zone, Northwest Ethiopia, November to December, 2018

Test		qPCR		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	% Kappa (95% CI)
		Pos	Neg					
Microscopy	Pos	105	2	77(70,83)	99(96,99)	98(91,99)	90(85,92)	0.74
	Neg	31	267					
PfHRP2 RDT	Pos	104	19	77(70, 83)	93(89, 95)	85(77, 89)	89(85, 92)	0.80
	Neg	32	250					
PfPLDH RDT	Pos	95	3	70 (62, 77)	98(96, 99)	97 (91, 99)	66(60, 70)	0.74
	Neg	41	266					

NB: Pos = Positive, Neg = Negative, CI = Confidence interval, PPV = Positive Predictive value, NPV = Negative Predictive value, qPCR = quantittive PCR

Discussion

The objectives of this study were to determine the performance of PfHRP2 and PfPLDH rapid diagnostic test for diagnosis of P.falciparum in clinical setting. Continuous monitoring the performance of malaria RDT in local setting is essential to support global effort of malaria control and elimination program[3]. Based on the WHO limit of detection of sensitivity (> 95%) and specificity (> 90), findings of this study indicated that PfHRP2 and PfPLDH RDT could be used for malaria diagnosis at health post and other public health facility levels in the absence of microscopy [31].

PfHRP2 RDT showed a high positivity rate of P. falciparum (30.3%; 123/406) and a high negative predictive value (99%) compared to microscopy. These results confirmed that PfHRP2 RDT has reasonable diagnostic performance at peripheral health centers to correctly identify malaria-free individual as true negative. Furthermore, high positive predictive value (84%) revealed a low false positive rate by pfHRP2 RDT. These findings are in line with previous studies conducted at Ethiopia[32], Kenya[33] and Ghana[34]. However, when compare to qPCR, the P. falciparum positivity rate of pfHRP2 RDT was much lower (33.5%; 136/405). This difference is likely due to the fact that PCR is a more sensitive and specific assays compared to RDTs to detect malaria parasite as supported by previous studies [35, 36]. The false-negative result by PfHRP2 RDT highlighted that considerable number of malaria-infected

patients were misdiagnosed. If these false-negative individuals remain untreated, they could serve as malaria reservoir patients and may fuel onward malaria transmission in the community[37, 38].

There was no significant association of both gender and age group with microscopic-based parasite density. Likewise, no significant association was observed between those demographic factors and infection rate which was detected by malaria RDT and qPCR. This finding is consistent with previous study[39]. However, it is not in line with study at Uganda [40].This might be due to the difference in the age group and study participant, clinical malaria suspected individual involved in study.

The sensitivity of PfHRP2 96% (95% CI, 91 to 99%)) was high in comparison with PfPLDH RDT 89% (95% CI, 81 to 93%) but the specificity of PfHRP2 93% ((95% CI, 90 to 96%)) was low in comparison with PfPLDH RDT 99% (95% [CI, 97 to 100%)) based on microscopy as reference method for clinical samples. The specificity of 99% in the PfPLDH diagnostic test in this study was in line with manufacturer report. A similar pattern was also observed in previous studies [41, 42]. Such a high specificity of PfPLDH RDT with reference to microscopy could minimize false positives results in clinical malaria detection [43–45].

The sensitivity of PfPLDH (89%) was lower than the sensitivity (98%) showed in the manufacture. The sensitivity of both PfHRP2 and PfPLDH RDT increased by the level of parasite density based on microscopy. This finding agrees with the WHO recommendation [31] and is in line with a number of previous studies [43, 46]. This study also showed lower sensitivity for both PfHRP2 and PfPLDH in comparison with qPCR from clinical samples. Genetic variation of Pfhrp2 and its prozone effect might be the cause of lower sensitivity of this malaria RDT, false negative PfHRP2, as reported in the past studies[47–49]. Lower sensitivity of PfPLDH RDT in this as well as several other studies [42, 50] merits more attention to enhance its sensitivity. Our study found slightly higher values of sensitivity and specificity of PfHRP2 in comparison with others study[51]. These differences might be explained partly by infection status and geographical characteristic of the study participants, the type and batch of test products, and the reference method used to compare the diagnostic methods[31, 36].

This study showed acceptable performance of PfHRP2 and PfPLDH RDTs in clinical malaria detection, but we were not able to examine the accuracy of PfHRP2 and PfPLDH RDTs among asymptomatic individuals in the community. Furthermore, the potential cause of false positive results, cross-reactivity with rheumatoid factor [52], and thermal stability during transportation and storage were not assessed [31].

In general, there was good agreement between these malaria RDT with the reference microscopy for diagnosis of clinical malaria in this study. However, the results of this study also indicate us there is the need of highly sensitive and specific diagnostic method for malaria suspected patient in clinical settings to reduce false negative and false positive result associated with malaria RDT in the era of malaria control and elimination.

Conclusion

The sensitivity and specificity of CareStart^{TM}PfHRP2 and PfPLDHRDT in this study were based on WHO limit of detection for routine diagnosis of clinical malaria. Hence, this Pf/PV (HRP2/PLDH) Ag Combo RDT could continue to be used in the absence of microscopy in this study area. Furthermore, combining the high sensitivity of PfHRP2 and high specificity of PfPLDH will improve accuracy of malaria diagnosis and case management. However, it is critical to consider current biological threat, gene deletion and genetic variation of pfhrp2, which can cause false negative PfHRP2 based RDT. Therefore, continuous monitoring the performance of malaria RDT in local and country wide setting is important to consider an alternative malaria RDT like PfPLDH RDT to support control and elimination of malaria in Ethiopia.

Abbreviations

HRP2: Histidine-Rich Protein 2; PLDH: parasite-specific Lactate Dehydrogenase; PfHRP-2 : *Plasmodium falciparum* Histidine-Rich Protein 2 ; PfPLDH: *Plasmodium falciparum* specific Lactate Dehydrogenase ; PVPLDH : *Plasmodium vivax* specific Lactate Dehydrogenase; RDT: Rapid diagnostic test; SPSS: Statistical Package for Social Sciences; WHO: World Health Organization ; CI: Confidence interval ; DBS : Dried blood spots ; PCR: polymerase chain reaction; qPCR: quantitative polymerase chain reaction ; PPV:Positive Predictive value; NPV: Negative Predictive value **;** FMOH **:** Federal ministry of health of Ethiopia

Declarations

Conflict of interest

The authors declare that they have no conflict of interests.

Authors' contributions

GS, LG, DJ, EL, **CD and** KL conceived and designed the study. GS collected and analyzed the data. GS and LG drafted the manuscript. Finally, all authors commented and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during in this study are included in this published article and available from the corresponding author on reasonable request.

Consent for publication

This study reports no individual person's data. There is no opposition to its presentation and/or publication. All authors have read and approved the manuscript for publication.

Ethical consideration

Ethical clearance was obtained from the institutional review board of Aklilu Lemma Institute of Pathobiology, Addis Ababa University before data collection (Ref No ALIPB/IRB006/2017/18). Permission was obtained from Benishangul-Gumuz regional Health Bureau. Information about the study, the objective of the study, the possible risks and benefits of the study were explained to the participants or their guardians using the local language. Figure prink blood samples were taken after obtaining written informed consent and assent from parents or guardian's in case of children. Malaria positives were treated by health workers based on national treatment guideline. Confidentiality was maintained throughout the study.

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Figures

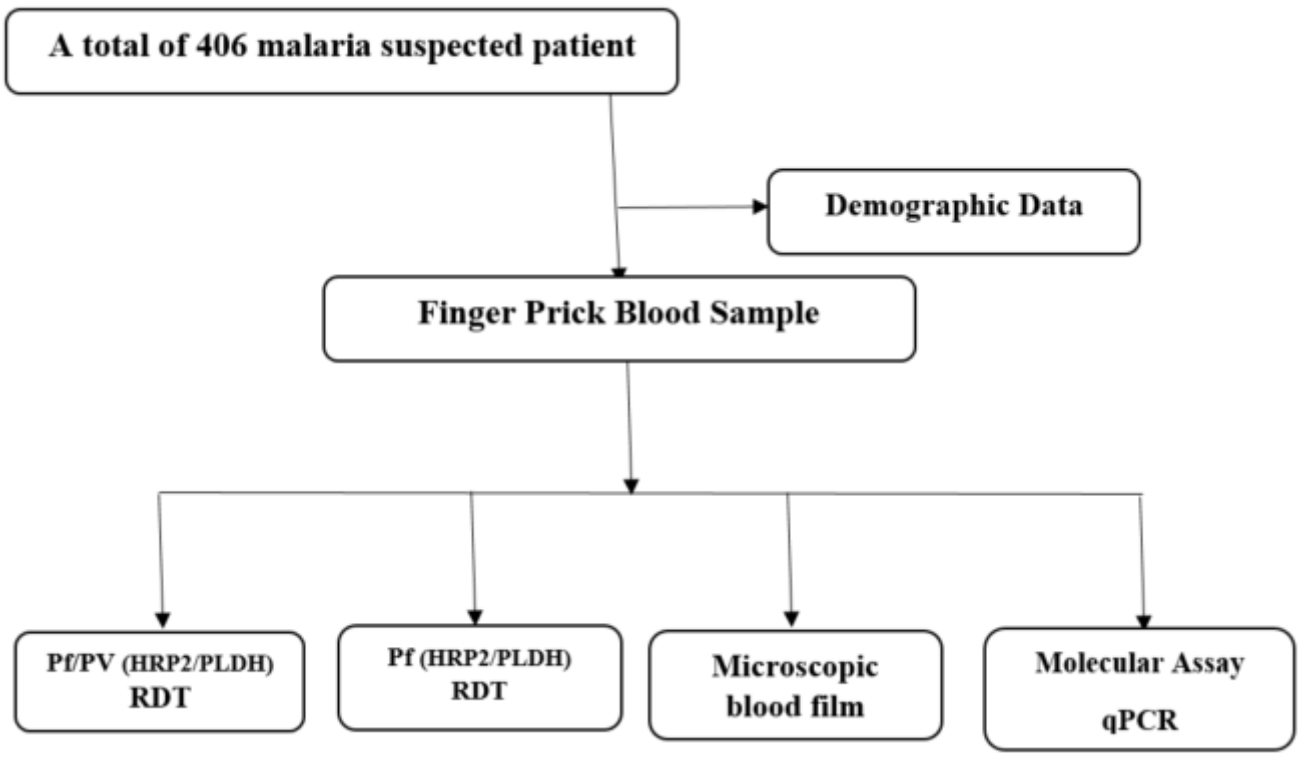

Figure 1

Study flow chart for Malaria RDT , Microscopy and Molecular assay