Plasmodium falciparum glutamate dehydrogenase is genetically conserved across eight malaria endemic states of India: Exploring new avenues of malaria elimination

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Abstract

Accurate and timely diagnosis is very critical for management, control and elimination of the malaria. Malaria rapid diagnostic tests (RDTs) have improved the diagnosis and management of malaria in remote areas, community and places where microscopy is not available for diagnosis. According to WHO report 2018, Plasmodium falciparum malaria constitutes more than 50% of malaria cases in India. Most of the RDTs used for diagnosis of falciparum malaria today employ HRP2 as a target antigen. However, low density parasitemia and deletion of hrp-2 gene in P. falciparum leads to false negative results and necessitates the development of alternative/ new or improved RDT for malaria diagnosis. We have analysed the genetic diversity and homology modelling of Pfgdh (glutamate dehydrogenase), ldh (lactate dehydrogenase) and aldolase genes in P. falciparum isolates from the eight endemic states of India to assess their potential as antigen for RDT development. We observed negligible sequence diversity in Pfgdh in comparison to the low level of diversity in ldh and aldolase gene. No structural or functional changes were observed in modelling studies and all three genes were under negative purifying selection pressure. The highly conserved nature of pfgdh gene suggests that GDH could be a potential target molecule for Pan/Pf diagnostic test for malaria.

Introduction

Malaria imposes heavy health and socioeconomic burden on tropical and sub-tropical regions of the world with an estimated 219 million cases and 435 000 deaths in 2017 [1]. India contributed about 85% of total malaria burden in the South East Asia Region [1]. Majority of malaria cases and deaths in India are reported from rural areas of the state with dominant tribal population. These include Odisha, Chhattisgarh, Jharkhand, Madhya Pradesh, Maharashtra, West
Bengal, Karnataka, Assam Gujarat and North-Eastern (NE) states [2]. In these states, malaria endemic districts are located in hilly, densely forested, remote, inaccessible areas and are often marred by inadequate health care facilities. Lackadaisical health care seeking behaviour and socio-economic backwardness add further salt to injury [3]. The WHO has set the goal of malaria elimination by 2030 [1]. However, this aim can only be achieved when all the indigenous cases of malaria are diagnosed correctly and treated properly through the available diagnostic tools like microscopy, Rapid Diagnostic Test (RDT) and Polymerase Chain Reaction (PCR). Microscopy is considered ideal for malaria diagnosis but it has several limitations including requirement of skilled microscopist for interpretation of results and poor sensitivity in detecting low-level parasitaemia [4]. With RDT, it is possible to diagnose malaria instantly in the field, rural and poor health care settings where microscopy is not available and clinicians have to make quick decision for case management. The ease of handling and prompt diagnosis has made malaria RDTs more popular. Since, its introduction, the sales of malaria RDTs has grown tremendously with distribution of 1.92 billion RDT globally during 2010–2017. About 276 million RDT were sold in 2017 alone in the world. Due to high prevalence of malaria, the WHO Africa region was on the biggest consumer of RDT (223 million) in 2017 [1]. The sales and use of RDT has increased from 10.6 million to 15.2 million with the total use of 118.1 million RDT units for malaria diagnosis during 2010–2017 in India, [1, 5].

**PfHRP2/3** based RDT are commonly used for diagnosis of falciparum malaria. **PfHRP3** is analogue of **PfHRP2** which share common antigenic epitope. At present out of 44 WHO recommended RDT, 41 RDTs target histidine-rich protein 2 (**PfHRP2**) either alone or in combination with **pfldh** for diagnosis of **P. falciparum** or pan Plasmodium genera [6]. Moreover, a number of studies from various parts of the world including India have reported the complete or partial deletion of **Pfhrp2/3** gene [7–9]. The deletion of **Pfhrp2/3** gene has rendered HRP2 based RDTs ineffective for diagnosis of such strains of **P. falciparum** and correlates with the poor performance of HRP2-based RDTs [10].

According to WHO report of Malaria RDT product testing few RDTs use Pf-pLDH for diagnosis of **P. falciparum** [6]. The sensitivity and performance of pLDH based pan malaria RDT and/or **P. vivax** pLDH RDT was found to be satisfactory. Very few RDTs (ParaHIT) use pan-Plasmodium aldolase in combination with **PfHRP-2** for diagnosis of malaria. The sensitivity of such RDTs were found to be poor because of relatively low expression of aldolase by the malaria parasite [11,12].

Concerns about the stability and performance of RDT (in terms of sensitivity and specificity) and antigen genetic diversity call for exploration and development of alternative or improved RDTs for malaria diagnosis [13–15]. Glutamate dehydrogenase (GDH) is considered a potential biomarker for **P. falciparum** diagnosis with high level of immunogenicity [16,17]. Glutamate dehydrogenase enzyme is a ubiquitous enzyme which plays important role in glutamate catabolism and ammonium assimilation [17,18]. It catalyses NAD(P)+ linked oxidative deamination reaction of l-glutamate to 2-oxoglutarate and ammonia. **P. falciparum** contains three genes encoding potential parasite glutamate dehydrogenase (pGDH) proteins; two genes are found on chromosome 14 (PF14_0164 and PF14_0286, encoding pGDH1 and pGDH2, respectively) and one on chromosome 8 (PF08_0132, encoding pGDH3). **P. falciparum** glutamate dehydrogenase (pGDH1) is the main producer of NAD(P)+ in erythrocytes and is essential for maintenance of redox balance of the cell [19]. Moreover, PFGDH is exclusively expressed by malaria parasite in erythrocytic stages and remain localised in cytosol [20] The concentration of GDH directly correlates with parasite load [17,19, 21]. Therefore, it can be used as marker of **P. falciparum**. To our knowledge very little or no data is available on genetic polymorphism of **pfaldolase**, **pfldh** and **pfdgh** gene in Indian isolates [22]. Therefore, the present research is intended on analysis of the sequence variation in Glutamate dehydrogenase.
gene, a potential diagnostic target for diagnosis of \textit{P. falciparum} and its comparison with lactate dehydrogenase and aldolase genes.

**Material and methods**

**Sample collection and study site**

The patient details and demographic information is already given in Bharti et al \cite{9}. Blood samples collected in the study to explore deletion of \textit{pfhrp2} and \textit{pfhrp3} gene in eight endemic states of India were used to determine the genetic diversity of \textit{pfgdh}, \textit{pfldh} and \textit{pfaldolase} genes (Fig 1). Ethical clearance was obtained from the Institutional Ethics Committee of National Institute of Research in Tribal Health (NIRTH), Jabalpur. Before collecting the samples, written informed consent was obtained from the patients or from the parents/guardian in case of children as per the guidelines of ICMR. Consent form was also provided and explained to the patients and parents/Guardian in case of children in local language.

**Genomic DNA isolation**

Parasite DNA was extracted from the whole blood samples of patients using QIAamp Blood kit as per manufacturer protocol and purified DNA was used as a template in PCR.

**Molecular diagnosis by Polymerase Chain Reaction**

Species-specific nested PCRs were performed in earlier study to confirm \textit{Plasmodium falciparum} mono-infection \cite{23}.

**PCR amplification of \textit{pfgdh}, \textit{pfldh} and \textit{pfaldolase} gene.** Positive samples for \textit{P. falciparum} were selected for the PCR amplification of \textit{pfgdh}, \textit{pfldh} and \textit{pfaldolase} gene using primers and PCR conditions described in Table 1. The primer were designed to amplify \textit{pfgdh} gene (PfGDH F1 and PfGDH R1), \textit{pfldh} gene (PfLDH F1 and PfLDH R1) and exon 2 of the \textit{Pf aldolase} gene (PfALDO F1 and PfALDO R1) as described in Fig 2, PCR reaction was carried out in a final reaction volume of 25µl reaction mixture containing 5µl of DNA template, 10X buffer, 0.4µM of each forward and reverse primers, 2mM \textit{MgCl}_2, 0.2mM each dNTPs and 0.2 units of \textit{Taq} Polymerase (Invitrogen, life technologies). PCR products of all genes were resolved on a 1.2% agarose gel and image was captured using GelDoc-It\textsuperscript{2} imager. The 1kb DNA ladder (invitrogen) was used to determine the size of PCR products.

**DNA sequencing and analysis**

Samples that showed the positive amplification for \textit{pfgdh}, \textit{pfldh} and \textit{Pfaldolase} genes were sequenced from both directions by using forward and reverse primers of respective genes. PCR products were purified by using spin columns (Real Biotech Corporation, Taiwan). The DNA sequencing of purified DNA was done using BigDye Terminator v3.1 Cycle Sequencing Kit on Applied Biosystems 3730 XL DNA analyser. Sequences were analysed using software v5.2 (Applied Biosystems) and were aligned using Bio Edit Sequence Alignment editor version 7.2.6.1 with the reference sequence of \textit{pfgdh}, \textit{pfldh} and \textit{pfaldolase} gene available in the database. Nucleotide sequences of poor quality were not included in the final analysis. Nucleotide sequences were further translated to amino acid using ExPASy translate tool (https://web.expasy.org/translate/). Amino acid sequences were aligned with the reference sequence to look for significant and non-significant structural/functional changes in protein.
The sampling of blood samples was done at selected site from the endemic district of respective states as indicated in map.

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Fig 1. Map of India showing sampling site and number of samples used in the study. The sampling of blood samples was done at selected site from the endemic district of respective states as indicated in map.
Table 1. Details of primer sequences, amplicon size and PCR conditions.

| Gene    | Primer Sequence (5’ 3’); Forward (F) and Reverse (R) | PCR product length | PCR conditions | No. of cycles |
|---------|-------------------------------------------------------|--------------------|----------------|---------------|
| pfgdh   | PFGDH_F1- TAAAGACAAAAACGGGAAGG PFGDH_R1- CGTTTCTCTTGTCCAATGTA | 1243bp             | 95˚C 30 sec    | 57˚C 30 sec | 72˚C 30 sec | 30 |
| pldh    | PFLDH_F1- ATGGCACCAAAGGCAAACAAATC PFLDH_R1- TTAAGCTAATGCCCTCATTCC | 951bp             | 95˚C 30 sec    | 58˚C 30 sec | 72˚C 30 sec | 30 |
| pfaldose| PFALDO_F1- AGCAGATGTGCGCGAAGAT PFALDO_R1- TTTCCTGGCATGTTCAAA | 924bp             | 95˚C 30 sec    | 57˚C 40 sec | 72˚C 30 sec | 45 |

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Fig 2. Schematic representation of primer binding site and location of (A) *P. falciparum* glutamate dehydrogenase (B) *P. falciparum* lactate dehydrogenase (C) *P. falciparum* aldolase gene in the chromosome.

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**Structural comparison**

The model of protein structure having polymorphic amino acid sequences were generated by template based modelling in SWISS-MODEL [24] and the model was aligned with the reference crystal structure available in protein data bank (PDB) using PyMOL molecular graphic system (v1.7.4.5) to assess any structural changes in 3D structure of protein [25]. Model quality was estimated using QMEAN score (Qualitative Model Energy Analysis) and the rmsd value (root mean square deviation) [26, 27].

**Statistical analysis**

Population genetic parameters were measured to quantify selection pressure using different methods in MEGA7 software [28]. Ratio of substitution rate at non synonymous and synonymous sites (dN/dS) was quantified using Nei and Gojobori (jukes—Contor) method [29, 30]. Average number of substitution (π) per site, heterozygosity per site (θ) per site and Tajima test statistic (D) were estimated [31,32]. The number of transitional and transversional substitutions per site from over all sequence pairs were also calculated using the Tamura-Nei model [33].

**Results**

A total of 514 microscopy and PCR positive samples (which were available in sufficient quantity) for *P. falciparum* were selected for present study from a previous PfHRP-2 study [9]. The sizes of *pfgdh*, *pfldh* and *Pfaldolase* amplified gene products were 1243bp, 924bp and 921bp respectively.

**Sequence analysis of gdh gene from *P. falciparum* Indian isolates**

A total of 360 successfully sequenced samples yielded good quality sequences. These sequences were compared with the reference sequence (PF3D7_1416500) available in the NCBI database. Only two nucleotide substitutions were observed in five samples at position C 546 G and T 672 C (Table 2). None of these nucleotide substitutions led to change in amino acid. Amino acid sequences showed 100% identity with *P. falciparum* 3D7 (XP_001348337.1). Additional information about homology analysis with other similar sequences is given in S1 Table.

**Sequence and structure analysis of ldh gene from *P. falciparum* Indian isolates**

A total of 450 successfully sequenced good quality sequences were analyse to assess genetic variation in *Pfldh* gene. The amplified *Pfldh* gene of 951bp code for 316 amino acids fragment. After comparing the nucleotide sequence with reference sequence (PF3D7_1324900), four single nucleotide substitution were detected in five different samples at A 123 G, T 159 C, A 765 G and G 814 A position (Table 2). Non synonymous substitution at 814 position leads to amino acid change from D to N (Fig 3). Rest of the samples showed 100% nucleotide homology with the reference sequence. Predicted model of polymorphic PfLDH haplotype were generated by user template model in Swiss Model workspace. The QMEAN score of model is 0.59. Both the models matched significantly with crystal structure of LDH from *P. falciparum* (PDB Id: 2A94) with RMSD value 0.056 Å (Fig 4). No significant alteration in 3D structure of protein was observed.
Sequence and structural analysis of aldolase genes from *P. falciparum* Indian isolates

A total of 464 samples were successfully sequenced and the sequences were aligned with the reference sequence (PF3D7_1444800) to analyse genetic variation in *Pf aldolase* gene. Eight single nucleotide substitutions were detected in eleven different positions: C 156 T, C 234 T, C 312 G, A 444 T, A 630 G, T 729 A, C 753 T and A 819 C (Table 2). Only one non synonymous substitution at 312 position from C to G leads to amino acid change from H to Q (Fig 3). Rest of samples showed 100% nucleotide homology with the reference sequence. Predicted PfALDO model of polymorphic haplotype was generated in Swiss Model workspace using template with the E-value 0.0, Max Identity 99% and Query Coverage 100%. Model quality was estimated using QMEAN score which is -0.86. Both the models matched significantly with crystal structure of aldolase from *P. falciparum* (PDB Id: 1A5C) with RMSD value 0.074 Å (Fig 5).

Table 2. Single nucleotide polymorphism and amino acid changes in *pfgdh*, *pfaldolase* and *pfldh* gene found in the study.

| S. No. | Gene                     | SNP (by position and change) | Amino acid change (by position and change) | No. of sample with SNPs / total no. of samples |
|-------|--------------------------|------------------------------|------------------------------------------|---------------------------------------------|
| 1.    | *P. falciparum* glutamate dehydrogenase | 546 (ACC to ACG) | Synonymous | 3/360 |
|       |                           | 672 (TCT to TCC) | Synonymous | 2/360 |
| 2.    | *P. falciparum* lactate dehydrogenase | 123 (CCA to CCG) | Synonymous | 1/450 |
|       |                           | 159 (GTT to GTC) | Synonymous | 1/450 |
|       |                           | 765 (AAA to AAG) | Synonymous | 1/450 |
|       |                           | 814 (GAT to AAT) | 272 (D to N) | 2/450 |
| 3.    | *P. falciparum* aldolase | 156 (AAC to AAT) | Synonymous | 1/464 |
|       |                           | 234 (TTC to TTT) | Synonymous | 1/464 |
|       |                           | 312 (CAC to CAG) | 104 (H TO Q) | 1/464 |
|       |                           | 444 (GCA to GCT) | Synonymous | 1/464 |
|       |                           | 630 (GCA to GCG) | Synonymous | 2/464 |
|       |                           | 729 (GCT to GCA) | Synonymous | 1/464 |
|       |                           | 753 (ACC to ACT) | Synonymous | 3/464 |
|       |                           | 819 (CCA to CCC) | Synonymous | 1/464 |

D, Aspartate; H, Histidine; N, Aspargine; Q, Glutamine.

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Fig 3. Multiple amino acid sequence alignment of *Paldolase* (XP_001348599.1), *Pfdh* (XP_001349989.1) and *Pfgdh* (XP_001348337.1) with test sequences using Clustal omega 1.2.4 multiple sequence alignment tool.

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Purifying selection pressure on \textit{pfgdh}, \textit{pfldh} and \textit{pfaldo} gene

The dN/dS ratio of \textit{pfldh}, \textit{pfaldo} and \textit{pfgdh} haplotype showed that all three genes are under negative selection pressure (Table 3). No transversion substitution was observed in \textit{pfldh} haplotype whereas transversion for \textit{Pfgdh} haplotype was higher as compared to the transitions.

Discussion

Diagnosis is critical for achieving the goal of malaria elimination set by the government of India by 2030. Rapid diagnostic tests (RDTs) are the major diagnostic tool particularly used in field and community surveys in malaria endemic countries with under-equipped health care infrastructures. The WHO has issued recommendations about the choice of RDTs, however selection of right RDT remains difficult for most users in endemic countries because of variation in targeted antigen in local parasitic population [34, 35]. One of the most important, but least studied of these factors is genetic variability of the target antigens of malaria parasite. [36, 37].

Most of the RDTs at present use pfHRP2, Plasmodial aldolase and Plasmodial lactate dehydrogenase as antigen for diagnosis of malaria parasite [38]. About 90% of RDTs targeting \textit{Plasmodium falciparum} use HRP-2 as antigen. Though the exact function of HRP-2 and its homolog HRP-3 are not clearly known but the protein is water soluble, heat stable, expressed...
in parasitophorous vacuole or cytoplasm of parasite [18]. The pfhrp-2 gene is located on chromosome 8 whereas pfhrp-3 is located on chromosome 13. It is not an essential gene and parasite can survive even when the both the genes are partially or completely deleted. The reports of partial or complete deletion of pfhrp-2/3 across the malaria endemic regions in world are worrisome [39]. The hrp-2 based RDTs fail to detect these parasites which continue to infect new hosts and cause malaria. The deletion of pfhrp-2 and prevalence of malaria parasite lacking the gene necessitates the development of alternative RDTs/tools based on other antigens for malaria diagnosis. Keeping in mind, we selected the *P. falciparum* positive blood samples from the same sites where hrp-2 deletion was detected [9] and the genetic diversity of pfldh, pfaldo

Fig 5. Structural alignment of predicted protein model of PfALDO with the reference crystal structure (PDB ID: 1A5C) in PyMol. Highlighted region in protein showing reference structure fully superimposed on the predicted protein structure of polymorphic haplotype with RMSD value almost 0.074.

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| Gene | No. of haplotype | dN | dS | dN/dS ratio | transition | transversion | S | θ | I | Tajima’s D |
|------|-----------------|----|----|-------------|------------|--------------|---|---|---|------------|
| pfldh | 4 | 0.0005 | 0.0057 | 0.087719298 | 0.0017 | 0 | 4 | 0.002019 | 0.001682 | -1.093799 |
| pfaldo | 8 | 0.0003 | 0.0061 | 0.049180328 | 0.0008 | 0 | 8 | 0.002652 | 0.0016 | -1.797517 |

Abbreviations: dN = Average number of nonsynonymous mutation, dS = Average number of synonymous mutation S = Number of segregating sites, θ = heterozygosity per site, π = nucleotide diversity, and D is the Tajima test statistic.

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and Pfaldo was analysed. Unlike the sequence polymorphism in pfhrp-2/3 reported by Bharti et al 2016 and 2017, pfaldolase and pfldh genes appear to be highly conserved. Aldolase is a glycolytic enzyme found in host tissues and malaria parasite. It shares 61–68% sequence similarity. The P. falciparum and P. vivax aldolase sequences are conserved and mostly employed for pan RDT as antigen. Likewise, out of eight nucleotide substitutions reported at different position of aldolase gene in this study only one substitution led to amino acid change (at codon 104). Despite having minor difference in the amino acid sequence, the structure of the protein did not change. However, Aldolase based RDTs are less popular because of low sensitivity due to less secretion of antigen [13,40].

In the absence of citric acid cycle, LDH is an important energy producing enzyme of glycolytic pathway of parasite. It is expressed in both sexual (gametocytes) as well as asexual stages of the parasite. Similar to aldolase, sequencing and comparative analysis of pfldh gene sequences showed 3 synonymous and 1 non synonymous (0.44%) mutations. Further, the amino acid sequence alignment of PfLDH and comparison of the protein structure with the reference in the PyMOL 1.7.4 shows that the 3D structure of PfLDH protein and region targeted in the RDT [40] remain conserved and the non-synonymous substitutions did not alter the enzyme structure. In contrast to our finding, Kelusker et al. reported no variability among sequences from a small number (n = 45) of Indian P. falciparum isolates collected from different study sites at Odisha, Karnataka and Goa [41]. However, the strength of our study is the analysis of at least 50–70 P. falciparum positive samples per site. A recent report about the analysis of genetic variation of pfldh gene from Thailand, India, Iran and Medagascar corroborates the conserved nature of pfldh gene [42]. However, ldh based RDTs are less sensitive. The poor sensitivity of LDH based RDTs is due to its inability in detecting low parasitemia and low sensitivity in high tropical temperature [43]. Further, recent sequence analysis from Thailand also supports our observation of conserved pfldh sequences.

Sequencing and analysis of pfgdh sequences shows that gene is highly conserved and only two synonymous SNPs were observed with no amino acid change among Indian isolates. Very little sequence diversity has been observed in PfGdh in comparison to the low level of diversity in ldh and aldolase gene. Similarly, Seol B and his colleagues also reported that gdh gene of P. vivax from South Korea are highly conserved [44]. The neutrality analysis of pfgdh, pfldh and pfaldolase genes using tajima D test shows that all genes are under negative purifying selection and are conserved. The homohexameric PfGDH protein (Mw = 49.5 kDa, GDH1 PF3D7_1416500) shares only 23% overall amino acid sequence identity with the human counterpart [45] (HGDH, Uniprot gene- GLUD1) where as 69–86% identity with Pv GDH (Uniprot gene-PVX_085005, PVX_085625), 86% identity with PmGDH (Uniprot gene—PMALA_027050), 85% identity with PoGDH (Uniprot gene - POVCU1_039950) and 85% identity with PkGDH (Uniprot gene- PKH_133070) (S1 Table). The similarity of PfGDH with other plasmodium species may lead to cross reaction in areas where mixed malaria infection are found [23]. Though some studies have reported the PfGDH concentration in serum of malaria patients including in asymptomatic malaria, usually lies in nanomolar range [46], yet further studies on GDH expression in local clinical samples from malaria patients are required to test the efficacy of GDH as potential biomarker. The major limitation of the study is our inability in correlating the level of parasitemia with that of expression of pfgdh, pfldh and pfaldolase genes and their antigen levels in blood samples due to retrospective nature of the study. Our study provides scientific evidence for the conserved nature of pfgdh gene/protein sequences in Indian isolates which can be used as a potential biomarker for diagnosis of malaria. A recently published study has reported the development of DNA aptamer based biosensor targeting PfGDH and PLDH gene [45,47]. However, validation of the device needs to be done in field conditions. In light of deletion of pfhrp2/3 genes and poor sensitivity of ldh and
aldolase based RDTs, it is imperative to explore the new diagnostic biomarker to supplement the arsenal of malaria diagnostics and to achieve the malaria elimination goal. This study creates basis for further evaluation of pfGDH based malaria diagnostics for future applications in pfhrp2/3 deleted areas.

Supporting information
S1 Table. Homology analysis of *P. falciparum* glutamate dehydrogenase.

(DOCX)

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References

1. World Health Organization (WHO), World malaria report 2018. who.int/iris/bitstream/handle/10665/275867/9789241565653-eng.pdf [cited 2019 Jan 14].

2. Sharma RK, Thakor HG, Saha KB, Sonal GS, Dhariwal AC, Singh N. Malaria situation in India with special reference to tribal areas. The Indian journal of medical research. 2015 May; 141(5):537. PMID: 26139770

3. Singh N, Singh OP, Sharma VP. Dynamics of malaria transmission in forested and deforested regions of Mandla District, central India (Madhya Pradesh). Journal of the American Mosquito Control Association-Mosquito News. 1996 Jun 1; 12(2):225–34.

4. Endeshaw T, Graves PM, Ayele B, Mosher AW, Gebre T, Ayalew F, et al. Performance of local light microscopy and the ParaScreen Pan/Pf rapid diagnostic test to detect malaria in health centers in Northwest Ethiopia. PloS one. 2012 Apr 20; 7(4):e33014. https://doi.org/10.1371/journal.pone.0033014 PMID: 22536317

5. National Vector Borne Disease Control Programme (NVBDCP) Strategic Plan for Malaria Control in India 2012–2017. [cited 2018 July 10] http://nvbdcp.gov.in/WriteReadData/692a/Strategic-Action-Plan-Malaria-2012-17-Co.pdf

6. WHO Malaria Rapid Diagnostic Test Performance, Results of WHO product testing of malaria RDTs: Round 7 (2015–2016) [cited 2018 August 18] who.int/iris/bitstream/handle/10665/255836/9789241512688eng.pdf?sessionid=34F105428A9A64DCB68A9683C08E877C?sequence=1
7. Gamboa D, Ho MF, Bendezu J, Torres K, Chiodini PL, Barnwell JW, et al. A large proportion of P. falciparum isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. PLoS one. 2010 Jan 25; 5(1):e8091. https://doi.org/10.1371/journal.pone.0008091 PMID: 20111602

8. Koita OA, Doumbo OK, Ouattara A, Tall JK, Konaré A, Diakité M, et al. False-negative rapid diagnostic tests for malaria and deletion of the histidine-rich repeat region of the hrp2 gene. The American journal of tropical medicine and hygiene. 2012 Feb 1; 86(2):194–8. https://doi.org/10.4269/ajtmh.2012.10-0665 PMID: 22302847

9. Bharti PK, Chandel HS, Ahmad A, Krishna S, Udhayakumar V, Singh N. Prevalence of pfhrp2 and/or pfhrp3 gene deletion in Plasmodium falciparum population in eight highly endemic states in India. PLoS One. 2016 Aug 12; 11(8):e0157949. https://doi.org/10.1371/journal.pone.0157949 PMID: 27518538

10. Baker J, McCarthy J, Gatton M, Kyle DE, Belizario V, Luchavez J, et al. Genetic diversity of Plasmodium falciparum histidine-rich protein 2 (PFHRP2) and its effect on the performance of PFHRP2-based rapid diagnostic tests. The Journal of infectious diseases. 2005 Sep 1; 192(5):870–7. https://doi.org/10.1086/432010 PMID: 16088837

11. Le Roch KG, Johnson JR, Flores L, Zhou Y, Santrosyan A, Grainger M, et al. Global analysis of transcript and protein levels across the Plasmodium falciparum life cycle. Genome research. 2004 Nov 1; 14(11):2308–18. https://doi.org/10.1101/gr.2623904 PMID: 15820293

12. Barber BE, Williams T, Grigg MJ, Piera K, Yeo TW, Anstey NM. Evaluation of the sensitivity of a pLDH-based and an aldolase-based rapid diagnostic test for the diagnosis of uncomplicated and severe malaria caused by PCR-confirmed Plasmodium knowlesi, Plasmodium falciparum and Plasmodium vivax. Journal of clinical microbiology. 2013 Jan 23; JCM-03285.

13. Perkins MD, Bell DR. Working without a blindfold: the critical role of diagnostics in malaria control. Malaria journal. 2008 Dec; 7(1):SS.

14. Bell D, Wongsrinchanalai C, Barnwell JW. Ensuring quality and access for malaria diagnosis: how can it be achieved?. Nature Reviews Microbiology. 2006 Sep; 4(9):682. https://doi.org/10.1038/nmmicro1474 PMID: 16912713

15. Rodríguez-Acosta A, Domínguez NG, Aquilar I, Girón ME. Characterization of Plasmodium falciparum glutamate dehydrogenase-soluble antigen. Brazilian journal of medical and biological research. 1998 Sep; 31(9):1149–55. https://doi.org/10.1590/s0100-879x1998000900008 PMID: 9876282

16. Wagner JT, Lüdemann H, Färber PM, Lottspeich F, Krauth-Siegel RL. Glutamate dehydrogenase, the marker protein of Plasmodium falciparum: cloning, expression and characterization of the malarial enzyme. European journal of biochemistry. 1998 Dec 1; 258(2):813–9. PMID: 9874251

17. Jain P, Chakma B, Patra S, Goswami P. Potential biomarkers and their applications for rapid and reliable detection of malaria. BioMed research international. 2014; 2014.

18. Vander Jagt DL, Hunsaker LA, Kibirige M, Campos NM. NADPH production by the malaria parasite Plasmodium falciparum. Blood. 1989 Jul 1; 74(1):471–4. PMID: 2665841

19. Vander Jagt DL, Intress C, Heidrich JE, Mrema JE, Rieckmann KH, Heidrich HG. Marker enzymes of Plasmodium falciparum and human erythrocytes as indicators of parasite purity. The Journal of parasitology. 1982 Dec 1:1068–71

20. Storm J, Perner J, Aparicio I, Patzewitz EM, Olszewski K, Llinas M, et al. Plasmodium falciparum glutamate dehydrogenase is dispensable and not a drug target during erythrocytic development. Malaria journal. 2011 Dec; 10(1):193

21. Zocher K, Fritz-Wolf K, Kehr S, Fischer M, Rahlf S, Becker K. Biochemical and structural characterization of Plasmodium falciparum glutamate dehydrogenase 2. Molecular and biochemical parasitology. 2012 May 1; 183(1):52–62. https://doi.org/10.1016/j.molbiopara.2012.01.007 PMID: 22342964

22. Farooq U, Dubey ML, Shrivastava SK, Mahajan RC. Genetic polymorphism in Plasmodium falciparum: Differentiation of parasite isolates of high & low virulence by RAPD. The Indian Journal of Medical Research. 2012 Aug; 136(2):292. PMID: 22960898

23. Krishna S, Bharti PK, Chandel HS, Ahmad A, Kumar R, Singh PP, et al. Detection of mixed infections with Plasmodium spp. by PCR, India, 2014. Emerging infectious diseases. 2015 Oct; 21(10):1853. https://doi.org/10.3201/eid2110.150678 PMID: 26401635

24. Waterhouse A, Berton M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic acids research. 2018 May 21.

25. The PyMOL Molecular Graphics System, Version 1.7.4.5 Schrödinger, LLC. http://pymol.org/ep/

26. Benkert P, Tosatto SC, Schomburg D. QMEAN: A comprehensive scoring function for model quality assessment. Proteins: Structure, Function, and Bioinformatics. 2008 Apr 1; 71(1):261–77.

27. Mairov NV, Crippen GM. Significance of root-mean-square deviation in comparing three-dimensional structures of globular proteins.
28. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution. 2016 Mar 22; 33(7):1870–4. https://doi.org/10.1093/molbev/msw054 PMID: 27004904
29. Nei M, Gojobori T. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Molecular biology and evolution. 1986 Sep 1; 3(5):418–26. https://doi.org/10.1093/oxfordjournals.molbev.a040410 PMID: 344411
30. Jukes TH, Cantor CR. Evolution of protein molecules. Mammalian protein metabolism. 1969; 3 (21):132.
31. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 1989 Nov 1; 123(3):585–95. PMID: 2513255
32. Nei M, Kumar S. Molecular evolution and phylogenetics. Oxford university press; 2000 Jul 27.
33. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular biology and evolution. 1993 May 1; 10(3):512–26. https://doi.org/10.1093/oxfordjournals.molbev.a040023 PMID: 8336541
34. World Health Organization: Recommended selection criteria for procurement of malaria rapid diagnostic tests. (cited 15.8.18) who.int/iris/bitstream/handle/10665/259870/WHO-CDSS-GMP-2018.01-eng.pdf?sequence=1
35. Houzé S, Hubert V, Le Pessec G, Le Bras J, Clain J. Combined deletions of pfhrp2 and pfhrp3 genes result in Plasmodium falciparum malaria false-negative rapid diagnostic test. Journal of clinical microbiology. 2011 May 4;JCM-00281.
36. Lee N, Baker J, Bell D, McCarthy J, Cheng Q. Assessing the genetic diversity of the aldolase genes of Plasmodium falciparum and Plasmodium vivax and its potential effect on performance of aldolase-detecting rapid diagnostic tests. Journal of clinical microbiology. 2006 Dec 1; 44(12):4547–9. https://doi.org/10.1128/JCM.01611-06 PMID: 17021060
37. Talman AM, Duval L, Legrand E, Hubert V, Yen S, Bell D, et al. Evaluation of the intra-and inter-specific genetic variability of Plasmodium lactate dehydrogenase. Malaria journal. 2007 Dec; 6(1):140.
38. Hopkins H, Kambale W, Kamya MR, Staedke SG, Dorsey G, Rosenthal PJ. Comparison of HRP2-and pLDH-based rapid diagnostic tests for malaria with longitudinal follow-up in Kampala, Uganda. The American journal of tropical medicine and hygiene. 2007 Jun 1; 76(6), 1092–1097. PMID: 17556616
39. Verma AK, Bharti PK, Das A. HRP-2 deletion: a hole in the ship of malaria elimination. The Lancet Infectious Diseases. 2018 Aug 1; 18(8):826–7. https://doi.org/10.1016/S1473-3099(18)30420-1 PMID: 30064667
40. Hurdayal R, Achilou I, Choveaux D, Coetzer TH, Goldring JD. Anti-peptide antibodies differentiate between plasmodial lactate dehydrogenases. Peptides. 2010 Apr 1; 31(4):525–32. https://doi.org/10.1016/j.peptides.2010.01.002 PMID: 20093160
41. Keluskar P, Singh V, Gupta P, Ingle S. Plasmodium falciparum and Plasmodium vivax specific lactate dehydrogenase: genetic polymorphism study from Indian isolates. Infection, Genetics and Evolution. 2014 Aug 1; 26:313–22. https://doi.org/10.1016/j.meegid.2014.06.004 PMID: 24953504
42. Simpalipan P, Pattaradilokrat S, Harnyuttanakorn P. Global sequence diversity of the lactate dehydrogenase gene in Plasmodium falciparum. Malaria journal. 2018 Dec; 17(1):16. https://doi.org/10.1186/s12936-017-2157-5 PMID: 29316927
43. Hendriksen IC, Mtove G, Pedro AJ, Gomes E, Silamut K, Lee SJ, et al. Evaluation of a PHRP2 and a pLDH-based rapid diagnostic test for the diagnosis of severe malaria in 2 populations of African children. Clinical infectious diseases. 2011 May 1; 52(9):1105–7. https://doi.org/10.1093/cid/cir143 PMID: 21467015
44. Seol B, Shin HI, Kim JY, Jeon BY, Kang YJ, Pak JH, et al. Sequence conservation of Plasmodium vivax glutamate dehydrogenase among Korean isolates and its application in seroepidemiology. Malaria journal. 2017 Dec; 16(1):3. https://doi.org/10.1186/s12936-016-1653-3 PMID: 28049479
45. Singh NK, Jain P, Das S, Goswami P. Dye coupled aptamer-captured enzyme catalysed reaction for detection of pan malaria and P. falciparum species in laboratory settings and instrument-free paper based platform. Analytical chemistry. 2019 Feb.
46. Li Y, Ning YS, Li L, Peng DD, Dong WQ, Li M. Preparation of a monoclonal antibodies against Plasmodium falciparum glutamate dehydrogenase and establishment of colloidal gold-immunochromatographic assay. Di 1 jun yi da xue xue bao = Academic journal of the first medical college of PLA. 2005 Apr; 25(4):435–8 PMID: 15837649
47. Singh NK, Thungon PD, Estrela P, Goswami P. Development of an aptamer-based field effect transistor biosensor for quantitative detection of Plasmodium falciparum glutamate dehydrogenase in serum samples. Biosensors and Bioelectronics. 2019 Jan 1; 123:30–5. https://doi.org/10.1016/j.bios.2018.09.085 PMID: 30308419