MedCrave
Step into the World of Research

Journal of Nanomedicine Research

Research Article

Open Access

CrossMark

# Malaria aptasensor based on electrochemical detection of recombinant plasmodium L dehydrogenase as a biosensor for falsiparium type

## Abstract

A specific aptasensor for a definite type of malaria parasite is created here. This biosensing method is based on electrochemical detection of (Lactate Dehydrogenase) LDH in blood samples. In this research we used a specific Aptamer for recombinant- falsiparium lactate dehydrogenase detection and a specific electrochemical aptasensor was fabricated by glassycarbone electrode (GCE). This biosensor has shown a good performance for accurate detection of rf-pLDH in blood samples, Electrochemical Impedance Spectroscopy (EIS) and amperometric method were used as detection method. NADH oxidation signal was used as chronoamperometric signal as a strategy. The detection 0.8nM and 0.2nM was obtained by EIS and chronoamperometric measurements.

**Keywords:** electrochemical aptasensor, malaria diagnosis, recombinant plasmodium lactate dehydrogenase, enzymatic detection, chronoamperometry

Volume 8 Issue 1 - 2022

Laleh Enayati Ahangar, Karim Movassaghi, Fatemeh Yaghoobi
University of Isfahan, Iran

**Correspondence:** Laleh Enayati Ahangar, Faculty of chemistry, University of Isfahan, Iran, Tel +98 3137932128, Email laleh.enayat@gmail.com

**Received:** October 18, 2022 | **Published:** November 03, 2022

## Introduction

Malaria infection causes about 300 thousand deaths per year (WHO). Malaria parasitism transmitted o human by bit of infected mosquitoes and make a lot of deaths mostly in children aged less than 5 years, According to the World Health Organization (WHO)'s 2016 World Malaria Report. A few as 10-100 plasmodium parasites per microliter of blood can make a significant illness or death. Therefore, a fast, sensitive and accurate method is necessary for malaria detection to reduce number of death. Several methods were reported for detection of malaria biomarkers and whole red-blood cell.[1,2] The lactate dehydrogenase enzyme of malaria parasite has more activity than 200 fold than same human enzyme that is a good biomarker for malaria diagnosis also Malaria parasitism in infected erythrocytes metabolise hemoglobin produce hemozoin that could be used as biomarker, Chen and coworkers reported two methods based on SERS for hemozoin detection as malaria biomarker. They synthesised silver nanoparticles then mixed with lysed blood sample and Raman measurement were done on mixed sample. The enhancement of Raman signal occurred because of contact of hemozine biomarker with nanoparticles.[1] In another research Peng and coworkers reported a label-free malaria diagnosis by using micro magnetic resonance relaxometry and hemozinthey studied the relatively paramagnetic susceptibility of hemozine, made by malaria parasites during intraerythocytic cycle, to the parasite load in blood. The magnetic susceptibility of hemozine induces changes in the transverse relaxation rate of proton nuclear magnetic resonance of red blood cell.[3] Also Varshney's group developed an label-free electrochemical method for detection of whole malaria infected red blood cells.[2]

Several methods were used for malaria diagnosis such as microscopy, spectroscopy[4,5] electrochemical[6–8] enzyme linked immunosorbent assay(ELISA),[9,10] immunochromatographic antigens antibody capture assay, surface enhanced Raman Spectroscopy(SERS),[11] quartz crystal microbalance(QCM), fluorescence antibody test(IFAT), droplet micro fluidics platform,[12,13] Laser light scattering,[14] Laser desorption mass spectrometry,[15,16] attenuated total reflectance infrared spectroscopy,[17] micromagneticres in ancerelaximetry(MRR),[3] computational study,[18] nanotechnology[1,19] and gas chromatography.[20] Electrochemical methods for malaria detection offer several advantages such as fast detection, simplicity, low price, high sensitivity, low microliter as sample volume, direct analysis of blood sample. A sensitive method that could detect parasitemia for positive samples below <50 parasite/ul is needed for detection of disease in first stage of infection and starting medical process .There are some biomarkers for this purpose histidin rich protein 2, parsite Lactate dehydrogenase (pLDH), hemozoin[1] cticpor 2017, tand aldolase.[1,21] Attarate's group developed new approach by DNA of malaria. Specific fragment of malaria DNA QCM were selected as probe and signal detection method. A biotinated 32-mer DNA was used to immobilize on transducer face.[22] Because of high activity of the lactetede hydrogenase enzyme of malaria parasite than same human enzyme makes it as a good biomarker. Different methods, based on pLDH detection have been developed for malaria diagnosis[21] that we pointed to some of them here.

Roa's search group developed an amperometric immunosensor by using gold nanoparticles/alumina sol-gel modified screen printed electrodes. They dripping $Al_2O_3$ sol on electrode surface then using electrochemical depositing method for produce gold nanoparticles on the $Al_2O_3$ modified electrode. Antibodies plasmodium falsiparumhestidine rich protein 2 complexes immobilised on the electrode.[23] Ban and co-workers reported an aptasensor to detect pLDH. They used cationic polymers and gold nanoparticles for colorimetric measurements. Poly (diallyl-dimethyl) ammonium chloride and poly (ally amine) in present f-pLDH will make aggregated AuNPs and changing the color from red to blue.[24] In 2014 same group used systematic evolution of ligands by Exponential enrichment (SELECX) for identification of a DNA aptamer with three regions i) loop 1ontains TTGG-T-G, ii) loop 2 contains AGT iii) loop 3: AG-C richtem. Electrochemical impedance was used for detection of both plasmodium vivax and plasmadiumfalsiparum LDH up to 1 pM.[6] In another research Ban's group used cationic surfactant, gold nanoparticles (AuNp) and DNA aptamer for colorimetric detection of malaria based on detection of pLDH. AuNp was used as probe and detection strategy was based on aggregation of AuNps in present of malaria pLDH.[5] LDH converts L-lactate into pyruvate and NAD to NADH and NADH has a oxidation peak around 0.4- 0.6 V depending the type of electrode and modification, The Measurement of enzymatic activity of pLDH could be done by electrochemical oxidation signal of (Nicotinamide adenine dinucleotide reduced form) NADH.[25]

Here, a label free specific aptasensor for a specific type of malaria parasite (rf-pLDH) is described. This biosensing method is based on electrochemical detection of (Lactate Dehydrogenase) LDH in blood samples. In this research we used a specific Aptamer for fabrication a specific electrochemical aptasensor by Glassy Carbone electrode (GCE) for accurate detection of rf-pLDH in blood samples, Electrochemical Impedance Spectroscopy (EIS) and chronoamperometric methods were used as detection method. NADH oxidation signal was used as chronoamperometric signal.

## Experimental

### Chemicals

N-hydroxylsuccinimide (NHS), carboxylicacid (FCA), N-(3-dimethylaminopropyl)-n-ethyl-carbodimideshydro-chloride (EDC), potassium ferrocyanide, potassium frricyanid, potassium chloride, sodium chloride, magnesium chloride, calcium chloride, phosphate salts, magnesium chloride. Lactic acid, Nicotinamide adenine dinucleotide oxidized form (NAD+), Nicotinamide adenine dinucleotide reduced form (NADH) and were purchased from Merck Company and Sigma Aldrich Company.

All solution preparation was done with the MilliQ water (18 MΩcm-1).

NH2- 5'-GCC TGT TGT GAG CCT CCT AAC (CAG CTC GTA GAA AAA AAA AGA TAT TGC TTC AAT TAT CTC CTC GCG TTC AAT TAA CCC AG) CAT GCT TAT TCT TGT CTC CC-3'

The stock solution of the Aptamer-oligonucleotides (40 µM) were prepared with PBS buffer solution contain 100 mM of MgCl2 divided to small adequate and kept frozen at -20oC. All experiment was performed at room temperature. Rf-LDH protein was purchased and stock solution 0.25 mg/ml were prepare in PBS buffer solution and was divided to small adequate and kept frozen at -20°C

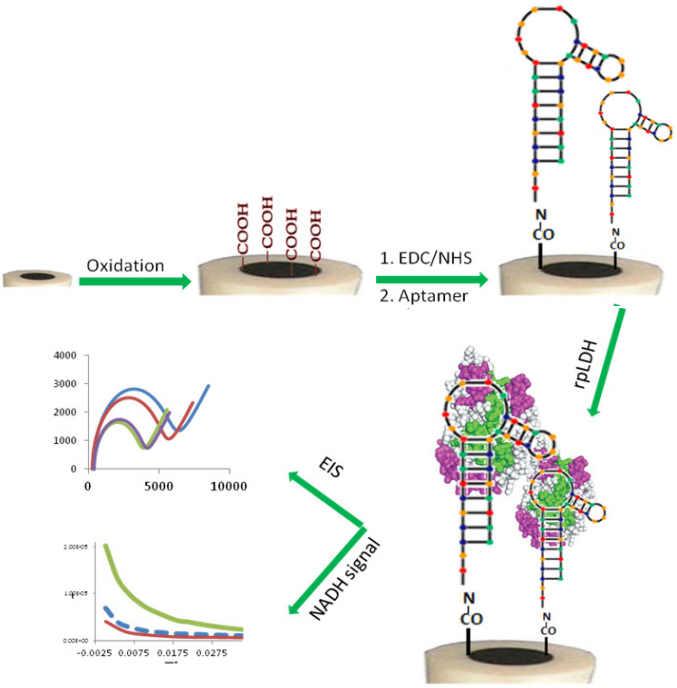
### Instrumentation

Electrochemical experiments were carried out using an Auto lab electrochemical system PGSTAT 302 [(Metrohm Auto lab B.V., Netherlands]. using NOVA, PGES 10 and FRA 10 software. All electrochemical measurements were done in a three electrode cell consisting of a glassy carbon working electrode (GCE, 3 mm diameter) from BioAnalytical Systems, as working electrode, a platinium counter electrode (ALS, Japan) and Ag|AgCl electrode (BioAnalysital Systems, 3 M KCl, saturated) as reference electrodes. The electrochemical impedance spectroscopy, differential pulse voltammograms have been recorded at the scan rate of 10 mVs-1 and the pulse amplitude of 25 mV. Cyclic voltamogrames was done in scan rate 50 MV.s-1. Electrochemical impedance spectroscopy (EIS), differential pulse voltammetry and cyclicvoltammetry (CV) experiments were performed with an AutolabPGSTAT302 (Metrohm Auto lab B.V., Netherlands) coupled to a Voltammetric Analytical stand (VA 663, Metrohm Auto lab B.V), Data analysis was performed using (GPES version 4.7.9, Eco Chemie B.V.), Frequency Response analyser (FRA, version 4.9.007, Metrohm Auto lab B.V.). Electrochemical Impedance Spectroscopy (EIS) experiments were accomplished at a constant DC potential of m the open-circuit potential (OCP) and the applied AC potential as 5mV, in the frequency range of 10 Hz–100 MHz. All experiments were performed at room temperature. A solution of 2mM potassium ferricyanide and potassium ferrocyanide (1:1) in 0.15 M KCl and 0.01 mMtris buffer pH 7.2 was used for EIS and CV measurements. For chroamperometric experiments a solution containing 0.2 M sodium lactare, 25 mM NAD+ , pH = 7.8 and potential 0.6V for 5 sec was used. The stock solution of the oligonucleotides (6 µM, from Eurofins MWG/Ope) was prepared two different Tris-buffer were used for fabrication of aptasensor, Tris-buffer 1 contains 20mM of tris-buffer, 300 mM of sodium chloride, 300 mM of potassium chloride, 200 mM of magnesium chloride and 100 mM of calsium chloride, pH 6.9. Tris-buffer 2 is similar to Tris-buffer-1 but pH:7.8 and used as binding buffer for best interaction of Aptamer and rf-pLDH.

## Procedures

### rf-pLDH-aptasensore preparation

Glassy carbon electrode was prepared as mentioned in literatures, briefly sequentially polished using on a Buëhler pad (BAS) with aluminium oxide slurry (<10 µm, Sigma–Aldrich) an -alumina slurry. Then it was rinsed thoroughly with the MilliQ water. Then the cleaned surface was oxidized with three linear sweeps from 0.7 to 1.15 V in PBS solution (pH 5.4) and it was rinsed. The generated carboxylic acid groups on the surface of GCE were activated by drooping 12 microliter of solution containing 5 mM EDC and 8 mM NHS in 1X PBS buffer pH5.5 for 1 h. Amino modified rf-pLDH-aptamer was covalently attached to the activated carboxylic groups as a result of dropping 7µ L of 4µM of aptamer solution plus 5 µ L of Tris-buffer-1. After 1 hour electrode surface was washed carefully by Tris-buffer-1. In next step some microliter of real sample or solution contain rf-pLDH and some microliter of binding buffer (totally 12 µl) were added to surface of prepared electrode for 40 min. after interaction time, the electrode surface was washed mildly with some droplet of binding buffer and immediately was used for electrochemical measurements. (Scheme 1)




**Scheme 1** Atasensor fabrication procedure.

### Real sample: whole blood samples

The concentration of parasites by EIS and chronoamperometric analysis. The preparation method was according Lee's method, briefly, 50 µ L of Real samples were mixed with 100 µ L of lysis buffer (150mM of Nh4Cl, 10mM of KNaCO3, 10 µM of EDTA) in a micro tube and shacked mildly for 15 min, then centrifuged 5 min at 3000 rpm, the supernatant was used for electrochemical

measurement. The original blood sample was diluted to reach lower concentration by serial dilution. Traditional microscopic analysis was used to determination of parasite concentration in malaria-infected blood samples. For selectivity test, Vivax-LDH was used at 25 nM.,

### EIS measurements

#### Chronoamperometric measurements

The enzymatic activity of rf-pLDH was used to produce electrochemical signal of present malaria aptasensors. The chronoamperometric signal of aptasensor at 0.6V, in 0.1M sodium lactate, 0.05M NAD+, pH =7.8 was used for Malaria detection signal.

## Results and discussion

### Characterisation of aptasensor surface during steps

The characterizations of the surface of modified GCE were done with CV and EIS techniques and a solution contains 2mM of potassium ferricyanide and 2 mM potassium ferrocyanide (1:1) in 0.1 M KCl.(Inset Fig. 1). By oxidation of GCE surface and producing –COOH groups on it, the [Fe(CN)6]3-/4- could reach to electrode surface harder because of negative charge of probe and negative charge of carboxylic groups. Therefore, the Ipeak for both, oxidation and reduction will have decreased in voltamograms and also Rct were increased in EIS results. (Inset Fig. 1). After activation and immobilizing of aptamer on the surface the barrier for charge transfer of probe with electrode surface will increase because of physical and repulsion of aptamer and electrostatic repulsion of negative charge of phosphate group of aptamer. (Figure 1) After combination of LDH to Aptamer because of physical repulsion the charge transfer barrier will increased therefore current decreasing in voltamograms and Rct increasing in EIS were observed respectively.

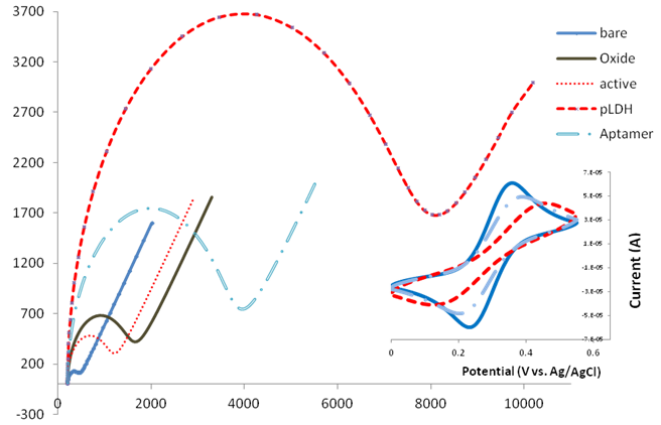



**Figure 1** EIS spectrum and inset CV behaviour of electrode surface modification step.

### Quantitative determination of Aptamer density

The Aptamer coverage on aptasensor surface was estimated by using Steel's methods, (steel 1998), briefly. RuHex as a cationic redox molecule was used. This molecule could electrostatically have absorbed on anionic phosphate group of immobilized aptameric, on modified electrode surface and the amount of cationic redox marker, RuHex, was measured by chronocoulometry method in the electrolyte with low ionic strength (0.02M KCl) to increase diffusion current at t0. The integrated charge, as a function of time t is explained by the integrated Cottrell expression, By using Cottrell Equation at t0 surface coverage of RuHex will be obtained as following equation:

$$\Gamma = Q / nFA_W$$

Γ :( Surface concentration of RuHex), Q: charge at t0, n: the number of electrons per molecule reduction (that here n is 1), F: Faraday constant, Aw: the electrode area ($cm^2$).

The real surface area of 3mM diameter, GCE was determined 0.071$cm^2$. Figure 2 is showing the results chronocoulometric experiment of 50µM RuHex adsorbed on biosensor surface at potential -0.3V and 0.02 M KCl electrolyte solution as blank on biosensor surface, As known 1 RuHex molecule could interact with 3 nucleotides (m=3) and the relation between numbers of adsorbed RuHex on DNA strands is describing with the following equation is showing:

$$\Gamma_{DNA} = \Gamma o\left(\frac{z}{m}\right)\left(N_A\right)$$

### Electrochemical response f aptasensor

The prepared aptasensor were incubating with different concentration of rPfLDH protein in optimized condition, then the electrochemical signal was followed by EIS measurement. Shown in Figure 2 IS plots in different concentration were optioned plot of Rct versus the rPfDLH concentration to achieve high sensitivity several parameters were optimized such as: buffer type, pH of buffer, oxidation process, binding buffer, time.

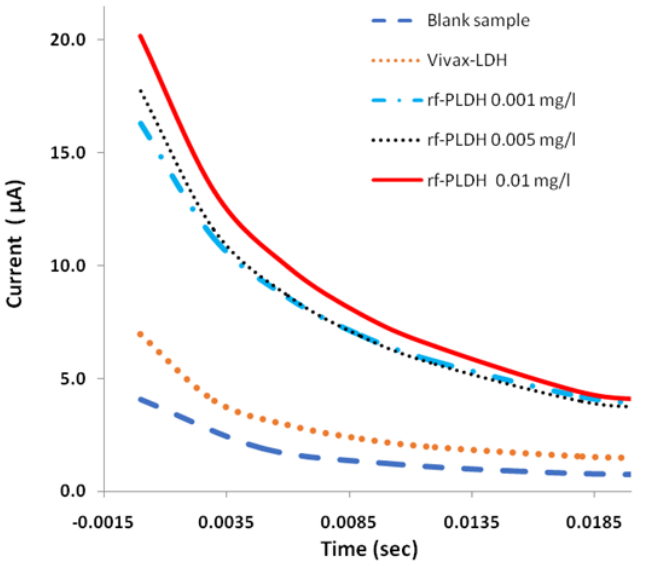



**Figure 2** EIS spectrum of Vivax pLDH, as blank and different concentration of rf-pLDH protein.

### Response of aptasensore real samples

The performance of electrochemical aptasensor was determined by incubation in infected blood samples and non-infected blood sample as control samples, The EIS analytical signals of the present aptasensor were determined in lysed malaria infected samples. The aptasensor was treated with real sample and control sample, and Apmerometic and electrochemical impedance spectra were recorded. Figure 1 show Nyquist plots of these biosensing procedures. That Rct values increase significantly in positive real sample from 3500 to 6500 kΩ, and for control samples it increased very slightly from 5500 kΩ to because of physical absorption of blood proteins. (Figure 3) The enzymatic activity of rf-pLDH was used to produce electrochemical signal of present malaria aptasensors were compared for control sample including the quantitative measurements of the different rPfLDH level as the Rct values is showed in Figure 4.

**Citation:** Ahangar LE, Movassaghi K, Yaghoobi F. Malaria aptasensor based on electrochemical detection of recombinant plasmodium L dehydrogenase as a biosensor for falsiparium type. *Int J Nanomed Res.* 2022;8(1):11–15. DOI: 10.15406/jnmr.2022.08.00202

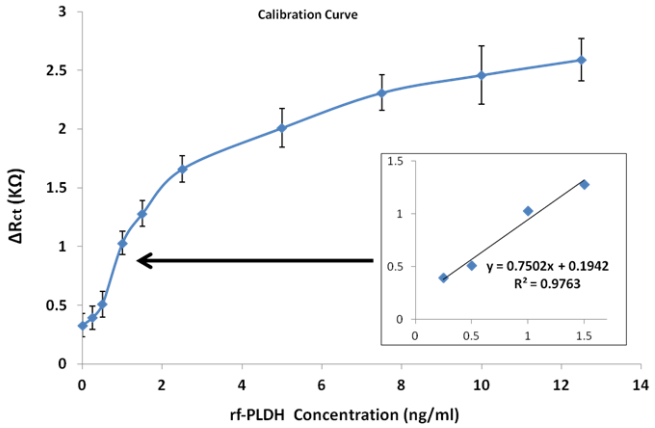



**Figure 3** Calibration curve of biosensor. (Plots of the relative increase of the $R_{ct}$ values in the Nyquist plot for different concentration of PfLDH protein).

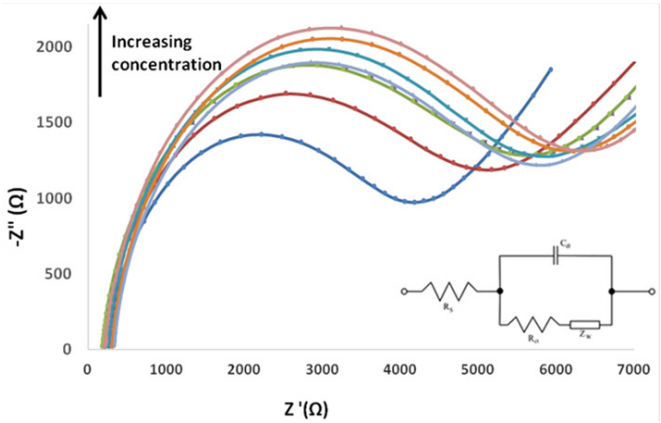



**Figure 4** The quantitative measurements of the different rPfLDH level.

## Conclusion

In this study a selective diagnosis Malaria aptasensors especially for recombinant - P. falciparum is created. The goal of selective detection of rPf-LDH was successfully achieved in real samples. The performance is compared to other similar / other methods in Table 1 the sensitivity, reproducibility and detection limit is comparable with other methods and selectivity is almost unique.

**Table 1** Comparison of present Aptasensor with previous methods for malaria detection

| Method | Strategy | Analyte | Detection limit | Ref. |
|---|---|---|---|---|
| Colorimetric detection | Cationic surfactant based using the Specific DNA Aptamer | PvLDH and PfLDH | 1.25 pM and 2.94 pM | 5 |
| Electrochemistry | EIS-aptasensor-gold electrode | p-vivax &p-falciparium | 1:00 PM | 6 |
| Antibody functionalized electrochemical biosensing | Gold nanoparticles enhanced platforms | PfLDH | <100 cell/ ml. | 2 |
| surface enhanced Raman spectroscopy | surface enhanced Raman spectroscopy (SERS) for hemozoin | PfLDH5 | 5nM 0.0005%, 100 parasites /µl | 1 |
| Quartz Crystal Microbalance (QCM) t | DNA segment of malaria | P. falciparum | 10 µg/ml | 22 |
| Surface enhanced Raman spectroscopy | based on silver nanorod array substrates | Plasmodium falciparum infected RBCs | Not Reported | 11 |
| Attenuated total reflectance infrared spectroscopy | Multivariate Analysis | Not mentioned | <1 parasit/µl | 17 |
| Electrochemical methods | EIS<br>Chronocoulometry | PfLDH | 0.8 nM<br>0.2 nM | present study |

## Acknowledgements

We gratefully acknowledge the support of this work by the Research Council of University of Isfahan and for supporting this research.

## Conflicts of interest

The author declares that there are no conflicts of interest.

## Funding

None.

## References

1. Chen K, Yuen C, Aniweh Y, et al. Towards ultrasensitive malaria diagnosis using surface enhanced Raman spectroscopy. *Sci Rep*. 2016;6:20177.
2. Kumar B, Bhalla V, Bhadoriya RPS, et al. Label-free electrochemical detection of malaria-infected red blood cells.*RSC Adv*. 2016;6(79):75862–75869.
3. Peng WK, Kong TF, Ng CS, et al. Micro magnetic resonance relaxometry for rapid label-free malaria diagnosis. *Nat Med*. 2014;20(9):1069–1073.
4. Samson EB, Goldschmidt BS, Whiteside PJD, et al. Photoacoustic spectroscopy of β-hematin. *J opt*. 2012;14(6):065302.
5. Lee S, Jeon W, Ban C, et al. Cationic Surfactant-Based Colorimetric Detection of Plasmodium Lactate Dehydrogenase, a Biomarker for Malaria, Using the Specific DNA Aptamer. *PLoS one*. 2014;9(7):e100847.
6. Lee S, Jeon W, Song KM, et al. A highly sensitive aptasensor towards Plasmodium lactate dehydrogenase for the diagnosis of malaria. *Biosens Bioelectron*. 2012;35(1):291–296.
7. Jain P, Das S, Chakma B, et al. Aptamer-graphene oxide for highly sensitive dual electrochemical detection of Plasmodium lactate dehydrogenase. *Anal Biochem*. 2016;514:32–37.
8. Sharma MK, Rao VK, Agarwal GS, et al. Highly Sensitive Amperometric Immunosensor for Detection of Plasmodium falciparum Histidine-Rich Protein 2 in Serum of Humans with Malaria: Comparison with a Commercial Kit. *J Clin Micro*. 2008;46(11):3759–3765.
9. Deraney RN, Mace CR, Rolland JP, et al. Multiplexed, Patterned-Paper Immunoassay for Detection of Malaria and Dengue Fever. *Anal Chem*. 2016;88(12):6161–6165.

**Citation:** Ahangar LE, Movassaghi K, Yaghoobi F. Malaria aptasensor based on electrochemical detection of recombinant plasmodium L dehydrogenase as a biosensor for falsiparium type. *Int J Nanomed Res*. 2022;8(1):11–15. DOI: 10.15406/jnmr.2022.08.00202

10. Gallien S, Roussilhon C, Blanc C, et al. Autoantibody against dendrite in Plasmodium falciparum infection: A singular auto-immune phenomenon preferentially in cerebral malaria. *Acta Trop*. 2011;118(1):67–70.

11. Chen F, Flaherty BR, Cohen CE, et al. Direct detection of malaria infected red blood cells by surface enhanced Raman spectroscopy. Nanomedicine: Nanotechnology. *Bio Med*. 2016;12(6):1445–1451.

12. Juul S, Nielsen CJF, Labouriau R, et al. Droplet Micro fluidics Platform for Highly Sensitive and Quantitative Detection of Malaria-Causing Plasmodium Parasites Based on Enzyme Activity Measurement. *ACS Nano*. 2012;6(12):10676–10683.

13. Kang YJ, Ha YR, Lee SJ. High-Throughput and Label-Free Blood-on-a-Chip for Malaria Diagnosis. *Anal Chem*. 2016;88(5):2912–2922.

14. Da Luz RAI, Mavoko HM, Crandallet I, al. Small angle light scattering assay for the detection of malaria infection. *Talanta*. 2016;147:473–477.

15. Demirev PA, Feldman AB, Kongkasuriyachai D, et al. Detection of Malaria Parasites in Blood by Laser Desorption Mass Spectrometry. *Anal Chem*. 2002;74(14):3262–3266.

16. Demirev PA, Mass spectrometry for malaria diagnosis. *Expert Rev Mol Diag*. 2004;4(6):821–829.

17. Khoshmanesh A, Dixon MWA, Kenny S, et al. Detection and Quantification of Early-Stage Malaria Parasites in Laboratory Infected Erythrocytes by Attenuated Total Reflectance Infrared Spectroscopy and Multivariate Analysis. *Anal Chem*. 2014;86(9):4379–4386.

18. Vyas S, Genis V, Friedman G, Computational study of a magnetic design to improve the diagnosis of malaria: 2D model. *J Magnetism Magnetic Mat*. 2017;423:301–305.

19. Brince Paul K, Kumar S, Tripathyet S et al. A highly sensitive self assembled monolayer modified copper doped zinc oxide nanofiber interface for detection of Plasmodium falciparum histidine-rich protein-2: Targeted towards rapid, early diagnosis of malaria. *Biosen Bioelec*. 2016;80:39–46.

20. Capuano R, Domakoski AC, Grasso F, et al. Sensor array detection of malaria volatile signature in a murine model. *Sen Actu Chem*. 2017;245:341–351.

21. Tangpukdee N, Duangdee C, Wilairatana P, et al. Malaria Diagnosis: A Brief Review. *K J Parasitol*. 2009;47(2):93–102.

22. Ittarat W, Chomean S, Sanchomphu C, et al. Biosensor as a molecular malaria differential diagnosis. *Clinica Chimica Acta*. 2013;419:47–51.

23. Sharma MK, Agarwal GS, Rao VK, et al. Amperometric immunosensor based on gold nanoparticles/alumina sol-gel modified screen-printed electrodes for antibodies to Plasmodium falciparum histidine rich protein-2. *Analyst*. 2010;135(3):608–614.

24. Jeon W, Lee S, Manjunatha DH, et al. A colorimetric aptasensor for the diagnosis of malaria based on cationic polymers and gold nanoparticles. *Anal Biochem*. 2013;439(1):11–16.

25. Rathee K, Dhull V, Dhull R, et al. Biosensors based on electrochemical lactate detection: A comprehensive review. *Biochem Biophys Rep*. 2016;5:35–54.

**Citation:** Ahangar LE, Movassaghi K, Yaghoobi F. Malaria aptasensor based on electrochemical detection of recombinant plasmodium L dehydrogenase as a biosensor for falsiparium type. *Int J Nanomed Res*. 2022;8(1):11–15. DOI: 10.15406/jnmr.2022.08.00202