Smartphone-Integrated Point-of-Care Sensor for Rapid and Sensitive Malaria Detection in Resource-Limited Settings

Meredith Stewart [1], Douglas Donaldson [1], Avni A. Argun [1], Muhit Rana[1] *

Abstract

Background: Malaria remains a major global health challenge, disproportionately affecting low-income countries and causing 450,000–720,000 deaths annually. Despite declining mortality rates due to containment strategies, the rise of drug-resistant *Plasmodium falciparum* threatens these gains. Current diagnostic methods, such as rapid diagnostic tests (RDTs) and microscopy, lack sensitivity, particularly in detecting asymptomatic infections. These undiagnosed cases contribute to ongoing malaria transmission, underscoring the urgent need for sensitive, cost-effective, and portable diagnostic tools for point-of-care (POC) use. This study aims to develop a low-cost, field-portable diagnostic device for rapid and sensitive detection of *Plasmodium* species, including *P. falciparum*, to enhance malaria intervention efforts in resource-limited settings. Methods: We designed an electrochemical sensor integrated with a mobile phone-based diagnostic platform. The device detects pan-*Plasmodium* and *P. falciparum* biomarkers in blood using enzyme-free amplification via SPAAC-mediated click chemistry. Four primer sets were optimized to target 18S rRNA sequences, and their sensitivity and specificity were evaluated. Chronoamperometric measurements were performed with a smartphone-linked portable potentiostat, enabling real-time data analysis and result reporting. Results: The 27/31 nucleotide primer set showed superior sensitivity and specificity. The assay achieved a detection limit of 100 pM with minimal off-target reactions. Chronoamperometric results were consistent with optical density measurements, validating the device's accuracy. The smartphone application effectively processed data for parasite quantification, though algorithmic refinement is needed to address minor inconsistencies. Conclusion: This low-cost and field-portable sensor demonstrates potential as a rapid, affordable, and sensitive tool for for point-of-care malaria detection in resource-limited settings. Its ability to identify low parasitemia levels can improve disease surveillance and intervention strategies. Future work will focus on optimizing the mobile application and conducting field validation studies to ensure reliability in diverse settings.

Keywords: Malaria diagnosis, Electrochemical sensing, *Plasmodium falciparum* detection, Point-of-care technology, Low-resource diagnostics

Significance | A low-cost, portable diagnostic device enables sensitive, rapid malaria detection, aiding intervention programs to reduce transmission and improve outcomes globally.

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1. Introduction

Malaria is a serious red blood cell infection caused by five species of single-celled eukaryotic *Plasmodium* parasites, which are transmitted to humans by Anopheles spp. Mosquitoes. One of these parasites, *Plasmodium* falciparum, is responsible for the deaths of ~1,200 African children under the age of 5 every single day (Holding & Snow, 2001). In some African countries the

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socioeconomic consequences of Malaria translate to billions of dollars every year (Gilles & Adetokunbo, 2002; Ouattara et al., 2011). In addition to exacerbating poverty and decreasing quality of life, frequent parasite exposure can negatively affect the growth, nutrition and brain development of children. *Plasmodium falciparum* is already the deadliest form of human malaria parasite, but threatens to pose an even larger global health burden with the emergence of antimalarial drug-resistant *P. falciparum* strains in Southeast Asia (Kihara, Carter, & Newton, 2006; Ouattara et al., 2011). Current methods to stop malaria transmission, like insecticides, bed nets, and artemisinin combination therapies (ACTs) for treatment, have resulted in a 60% global decline in malaria-caused deaths from 2000 to 2015((WHO), 2015b). ACTs are the most effective antimalarial medicine, combining fast-acting artemisinin-based compounds with a quinine derivative drug that acts against the parasite((WHO), 2015a). However, some strains of *Plasmodium falciparum* have acquired resistance to this treatment method due to Kelch 13 mutations and/or a gene duplication that renders the drugs used in ACTs, like piperaquine and mefloquine ineffective (Ariey et al., 2014; Haldar, Bhattacharjee, & Safeukui, 2018; Imwong et al., 2017; Noedl et al., 2008; Rossi, De Smet, Khim, Kindermans, & Menard, 2017).

Malarial diagnostics can not only be inaccessible to the very communities that need them but can also be characteristically unreliable due to the undetectable nature of many malaria infections. *Plasmodium* parasites can be present in asymptomatic individuals, as well as those that live in remote areas where testing is not readily available. Current rapid diagnostic tests (RDTs) detect antigen proteins from *P. falciparum* in the blood from a finger prick((WHO), 2016). But, according to WHO, these tests lack the necessary sensitivity to detect parasite nucleic acid biomarkers, especially in individuals with low parasite density. In addition to false positives, recent studies have shown that RDTs missed 60-75% of asymptomatic *Plasmodium* infections (Imwong et al., 2015; Wu et al., 2015). The limit of detection for routine light microscopy blood smear screening is 50,000 parasites per milliliter, whereas more sensitive PCR methods can detect parasitic infections down to 22 parasites per milliliter((WHO), 2016; Imwong et al., 2016; Joanny, Lohr, Engleitner, Lell, & Mordmuller, 2014). The availability of a more sensitive test is needed to detect parasites, especially those that persist in blood at low levels, to decrease malaria transmissions and for intervention strategies to be effective.

We report a low-cost, handheld POC diagnostic device for rapid, sensitive and selective detection of Malaria parasite infection in blood. The electrochemical sensing technology uses SPAAC-mediated enzyme-free amplification to detect two clinically validated biomarker sequences. Our innovative MalariaSENS™ will test for malaria infection in blood samples as well as the presence of the *P. falciparum* strain. MalariaSENS™ is a low-cost, portable sensor that connects via Bluetooth to a mobile device to report test results with a sample-to-answer turnaround time of 1 hour or less. This device will assist in the efforts to reduce Malaria *Plasmodium* parasite transmission and will be especially useful in low-resource areas, that often suffer the most from this disease.

2. Materials and Methods

2.1 *Materials and Equipment*

All the oligonucleotide primer sets (P1-P4) were purchased from Integrated DNA Technology, Inc, USA (IDT). The 20X PBST was purchased form Thermo Fisher (Catalog #28352). The TMB Super Sensitive One Component HRP substrate was purchased from Surmodics (Catalog #TMBS-0100-01). The streptavidin-coated plate was purchased from Pierce (Catalog #15120). DMSO (Catalog #472301-1L), Tween20 (P9416-50ML), and adult Bovine Serum (Catalog #B9433) was purchased from Millipore-Sigma. The Carrier DNA (Catalog #112200205) was purchased from MP Biomedicals. All other reagents were purchased from Sigma Aldrich.

2.2 Methods

2.2.1 Stock Preparations

Before beginning the experiment, the biosafety cabinet was cleaned with RNase Away. A dilution series using *P. falciparum* RNA as a positive control was prepared in bovine serum. The positive control target stock was combined with KCL in TE buffer at 25 nM, 2.5 nM, 250 pM, 25 pM and 2.5 pM concentrations. The carrier DNA was prepared at 750 µg/mL by combining 1.5 µL of 10mg carrier DNA with 18.5 µL of bovine serum. Next, 600 µL of carrier DNA in bovine serum was prepared at 1.25 µg/mL, 2.5 µg/mL, and 12.5 µg/mL.

2.2.2 Primer Design

We developed four separate primers to detect the 18s rRNA sequence, which was selected based on the work of our collaborator, Dr. Sean C. Murphy. The melting temperatures of each primer sequence were analyzed using IDT's Oligo Analyzer Tool™. The proximity of azide and DBCO reactive groups on the primer 3' and 5' ends lead to spontaneous ligation. Ligated primer sequences, containing both biotin (3') and digoxigenin (DIG) (5') labels can then be captured either on streptavidin-coated plates (Pierce). The reaction buffer for these specific primers required the presence of a non-specific protein to inhibit off-target reactions, KCl to neutralize the negative phosphate backbone and promote DNA hybridization, ammonium sulfate ((NH4)2SO4) and dimethyl sulfoxide (DMSO) to enhance target specificity, and Tween 20. Primers were tested under the following annealing temperatures: 66°C/31°C, 68°C/33°C, 70°C/35°C, 75°C/40°C. The sequence information for each primer can be found in Table 1.

2.2.3 Improving Assay Sensitivity

To test the limits of sensitivity for each of three different reactive groups (DBCO, DIBO and OCT), the 27/31nts primer set was evaluated under each condition. Samples were thermocycled 50 times under the following conditions: 66°C for 30 seconds followed by 31°C for 60 seconds (STT) or 66°C for 20 seconds followed by 31°C for 45 seconds. Samples were tested in 25% bovine serum with 1X reaction buffer (50 mM KCl, 20 mM ammonium sulfate, 5% DMSO, and 0.1% tween 20), with target DNA diluted to 1000 pM, 100 pM, 10 pM, and 0 pM.

2.2.4 Plate Imaging

A 96 well round bottom plate (Costar #7007) was used for the Epoch Plate Reader (Bio-Tek). Each well represented a unique combination of carrier DNA and target solution at different concentrations. Each well contained 20 µL of carrier DNA in bovine serum, 20 µL of target DNA, and 10 µL of each primer (2 plates needed). Plate 1 was run through the thermocycler at 68°C x5s /33°C x15s and plate 2 was run through the thermocycler at 98°C x5s /33°C x15s.

2.2.5 SPAAC-Ligation

After the first round of heating the primer pairs were initially denatured. The samples in each plate were spun down using the 5340R centrifuge at 4,680 rpm for 7.5 minutes and then the plates were flipped and spun again. In a new streptavidin-coated microtiter plate, 80 µL of 1% bovine serum were added to each well. 20 µl of each sample from plates 1 and 2 were added to a new corresponding well on the streptavidin coated plate. The plate is incubated at room temperature (RT) for 30 minutes while being shook at 500 rpm. To wash away the unbound sample, the plate was washed with PBST 3 times using the STD WASH 300X3 #17 plate washing program, which had been primed with 1X PBST. After the wash was completed, the OVERNIGHT LOOP #10 program on the automatic plate washer machine was run.

2.2.6 Antibody Detection

The anti-DNP-HRP-linked antibody (Detection antibody) was prepared in bovine serum at a 1:12,500 dilution with a final volume of 12 mL. 100 µL of the resulting antibody solution was added to each well of the streptavidin coated plate. The plate was then incubated at room temperature for 1 hour, being shook at 500 rpm. After, the plate was washed 3 times with PBST: first STD WASH 300X3 #17, followed by DAY RINSE #8, and finally RINSE AND SOAK #9.

12 µL of TMB Super Sensitive One Component HRP substrate was incubated at room temperature for one hour, away from light. The incubated TMB solution was then added to each well at 100 µL per well and incubated again at 10 minutes and 500 rpm, again, away from light. The reaction was stopped by adding 100 µL of 1M H2SO4 to every well. The plate was read on a plate reader at 450 nm.

3. Results and Discussion

The electrochemical sensor we developed uses enzyme-free SPAAC ligation to detect two clinically validated Malarial biomarker sequences, known as 18s rRNAs. To amplify the target DNA sequence, we developed four separate primer sets (P1-P4) from *Plasmodium falciparum* (Table 1). Once P1 and P2 have bound to the complimentary target sequence, DBCO and azide groups on the primers react, resulting in primer ligation (Figure1). After another round of heating and cooling, the P3 and P4 primers underwent ligation after binding to P1 and P2. We utilized a streptavidin coated microtiter plate to capture the primer complex and the anti-DIG-HRP-linked antibody.

3.1 Primer Selection

Of the four primer sets, 27/31 nts showed the best results with detection of 18s rDNA under every temperature condition (Figure S1). Detection was observed across almost all the buffer conditions tested. For this reason, we determined that the 23nts primer set was the most effective of the three sets.

3.2 Enhancing Sensitivity

We tested alternative click chemistries against the standard DBCO reaction group in order to optimize the sensitivity of the assay. We found that DIBO, an alternative dibenzocyooctyne, and OCT (cyclooctyne) are less reactive than DBCO, and therefore more likely to tolerate assay conditions (Figure S2). The 23/31 nts primer set was used to evaluate these chemistries even further. Both the DBCO and DIBO labeled primers produced high off target reactivity. However, the OCT labeled primers showed no off-target reactivity in the absence of a target (Figure S3). These results indicate that the OCT reaction chemistry is resistant to assay conditions that would otherwise prevent target detection with other primers.

3.3 Demonstrating Electrochemical Detection

Prior to finalizing assay conditions, we screened the 27/31 nts OCT labeled primers against different conditions and used chronoamperometric data to determine the best conditions (Figure 2). Chronoamperometric data generated in this experiment was captured using a portable, Bluetooth®-enabled potentiostat (PalmSens) provided to us by our collaborators at Enginasion. 18s rDNA oligo was tested in triplicate at concentrations ranging from 8000 pM – 3.9 pM in 25% bovine serum with 100 mM KCl, 20 mM ammonium sulfate, 5% DMSO, and 0.1% tween20. Samples were thermocycled at 90°Cx30 seconds/40°Cx60 seconds for 20 cycles. OD and chronoamperometry measurements were similar in terms of sensitivity and dynamic range. Chronoamperometric measurements were collected by measuring each sample at 250 mV for 30 seconds with measurements collected every 0.1 sec. Data was captured using PS Trace v 5.8 (PalmSens) and processed by averaging the values collected from 20 to 30 sec.

Table 1. Primer Sets for the Detection of Plasmodium falciparum 18s rRNA

ID	Primers (5' - 3')	NT	5' Label	3' Label	T_m
Set1_P1	A A T A A A T G T A T A G T T A C C T A T G T T C A A	27	DBCO	DIG	46.8
Set1_P2	T T T T A A T A A A A A T A T C C T A A T T T G A T T A C T G	31	NA	Azide-3'	54.8
Set1_P3	T T G A A C A T A G G T A A C T A T A C A T T T A T T	27	NA	DBCO	46.8
Set1_P4	C A G T A A T C A A A T T A G G A T A T T T T T A T T A A A A	31	Azide	Biotin	54.8
Set1_P1	A T C C T A A T T T G A T T A C T G A A T A A A T G	26	DBCO	DIG	48.0
Set1_P2	A G G G A A A A G G A T A T T T T A A T A A A A A T	26	NA	Azide-3'	47.8
Set1_P3	C A T T T A T T C A G T A A T C A A A T T A G G A T	26	NA	DBCO	48.0
Set1_P4	A T T T T T A T T A A A A T A T C C T T T T C C C T	26	Azide	Biotin	47.8
Set1_P1	G T T C A A T T T C A A A T A A G A A T A T A G T G T A C T C G C	33	DBCO	DIG	55.0
Set1_P2	A T C C T A A T T T G A T T A C T G A A T A A A T G T A T A G T T A C C T A T	39	NA	Azide-3'	55.2
Set1_P3	G C G A G T A C A C T A T A T T C T T A T T T G A A A T T G A A C	33	NA	DBCO	55.0
Set1_P4	A T A G G T A A C T A T A C A T T T A T T C A G T A A T C A A A T T A G G A T	39	Azide	Biotin	55.2
Set1_P1	A T C C T A A T T T G A T T A C T G A A T A A A T G T A T A G T T A C C	36	DBCO	DIG	54.2
Set1_P2	A T T A T T A G T A G A A C A G G G A A A A G G A T A T T T T A A T A A A A A A T	40	NA	Azide-3'	55.1
Set1_P3	G G T A A C T A T A C A T T T A T T C A G T A A T C A A A T T A G G A T	36	NA	DBCO	54.2
Set1_P4	A T T T T T A T T A A A A T A T C C T T T T C C C T G T T C T A C T A A T A A T	40	Azide	Biotin	55.1

(Top) Four sets of primers were purchased for use in this program. Each set is specific to P. falciparum 18s rRNA or rDNA. Each primer set utilizes DBCO/Azide reactions for SPAAC ligation and each set has different melting temperatures. (Bottom) A 112 NT ssDNA sequence matching P. falciparum 18s rRNA was used as a positive control for assay development purposes.

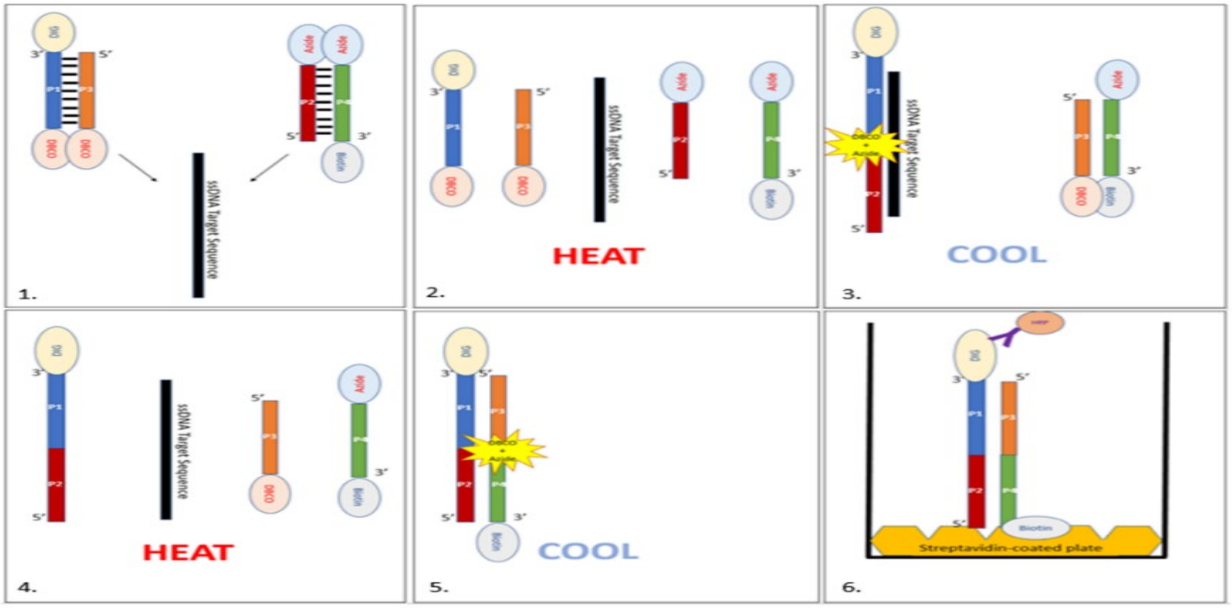


Figure 1. SPAAC-Ligation Assay Method.

Step 1. Pairs of labeled double-stranded primers are combined with a sample containing a target DNA sequence. Primers 1 and 3 (P1 and P3) carry DBCO groups on the 5' and 3' ends, respectively. P2 and P3 carry azide groups on the 3' and 5' ends, respectively.

Step 2. Heat is applied to the sample which results in denaturation of primer pairs.

Step 3. Sample is cooled to a temperature at which complementary sequences can anneal. Both P1 and P2 primers can bind to the complementary target sequence, which brings the reactive groups DBCO and azide into close proximity, resulting in primer ligation.

Step 4. Sample is heated again, resulting in denaturation of annealed P1-P2 primer from the target sequence.

Step 5. Sample is cooled, allowing target sequence to template a second pair of P1 and P2 primers. Ligated P1-P2 primer can now template P2 and P4 primers, resulting in a second round of ligation. Ligation product increases exponentially with each subsequent round of thermocycling.

Step 6. Upon completion of the thermocycling program, reacted sample is removed from the thermocycler and added a streptavidin-coated microtiter plate, which can capture the primer complex via the biotin tag on 3' end of the P3-P4 primer. Unbound sample is washed away, and reacted product is detected using an anti-DIG-HRP-linked antibody which binds the DIG tag on the 3' end of the P1-P2 primer.

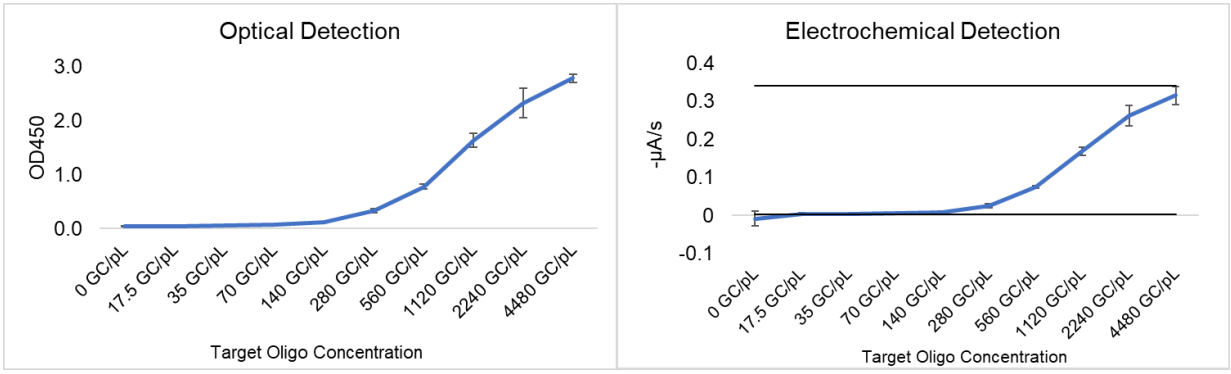


Figure 2. Comparison of Optical Density and Chronoamperometric Sample Measurements. 27/31nts OCT-labeled primers were tested at 90°C/40°C STTx20C in 100 mM KCl with standard buffer components and then plate data was read using both a plate reader to collect optical density measurements (left) and a portable potentiostat (PalmSens) (right). Solid black lines in the right-hand figure indicate upper and lower asymptotes as defined by a 4-parameter curve fit.

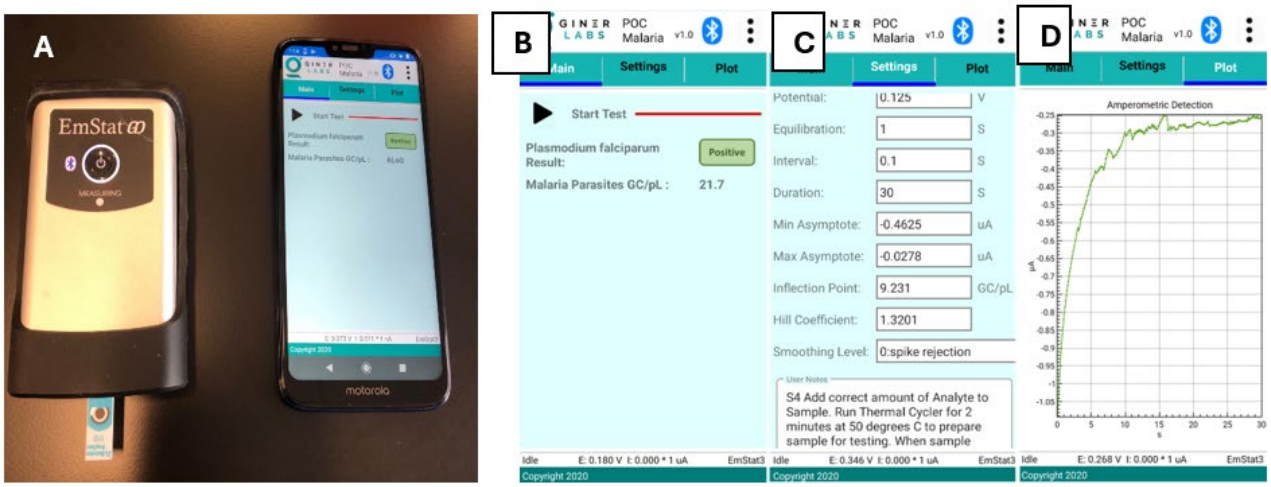
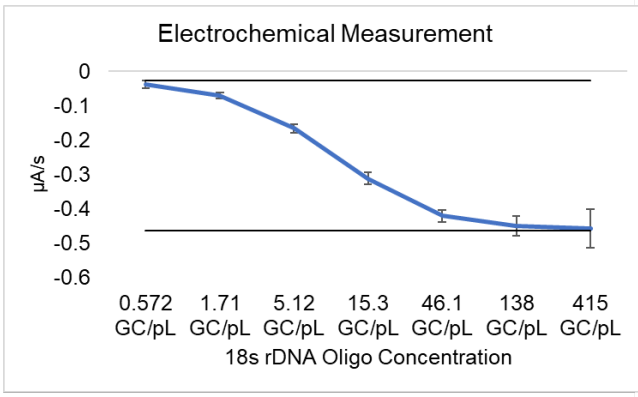


Figure 3. Giner Smartphone Application for the Detection of P. falciparum 18s rRNA. A) PalmSens potentiostat shown side by side with the app-enabled Android smartphone. B) The main screen is used to initiate sample testing and to report the result in GC/pL. C) The settings menu is used to input measurement parameters as well as standard curve parameters. D) The plot tab shows the resulting chronoamperometric data. The result is interpreted from the mean of the final 10 seconds of the measurement.



Parameter	Value
Inflection Point	9.231
Equation	$Y = -0.4625 + \frac{-0.0278 - 0.4625}{1 + (\frac{X}{9.231})^{1.3201}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{Inflection\ Point})^{Hill\ coefficient}}$

Figure 4. Electrochemical Measurement of 18s rRNA Oligo. A triplicate dilution series of 18s rDNA oligo was prepared and tested using the previously described ETTx50C-150mM KCl method. Sample values were captured using PSTrace v.5.8 (PalmSens) (left). The values of a 4-parameter standard curve were calculated using the website https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator. Minimum and maximum asymptote values as well as the inflection point, and Hill coefficient were used to define the standard curve range in the Giner smartphone application.

Chronoamperometric data generated in this experiment was provided to Enginasion to generate a mobile phone application capable of interpolating chronoamperometric data into a simple readout value. Since our goal was to determine the number of gene copies in a sample, we converted picomolar values to gene copies (GC)/picoliter (pL) using the following equation:

$$GC = \frac{(ng\ target\ x\ 6.022\ x10^{23})}{112\ x\ 1x10^{9}x\ 330\ g/mole}$$

In order to ensure that data could be interpolated from a set of standard measurements, we asked Enginasion to utilize a 4-parameter curve-fitting equation:

$$Y = Min + \frac{Max - Min}{1 + \left[\frac{X}{(inflection\ point)}\right]^{Hill\ coefficient}}$$

3.4 Mobile Phone Integrated Detection Device

Based on our electrochemical measurements (Figure 2), our collaborator Enginasion, built a smartphone application for malaria detection that pairs to a portable PalmSENS potentiostat. Once a sample is placed on the Bluetooth enabled potentiostat, the app calculated an average of the chronoamperometric measurements and generates a curve whose parameters are entered by the user. Screenshots of the results captured by the application are shown in Figure 3. The detection capabilities of this device were evaluated with a dilution series of the target DNA in bovine serum at concentrations 20 nM to 12.5 fM. The chronoamperometric measurements were collected by measuring each sample at 125 mV for 30 seconds, with a measurement collected every 0.1 sec. PS Trace v 5.8 (PalmSens) captured the data, and once values were averaged, a standard curve and 4-paramter curve fitting equation was obtained for values 0.572 GC/pL to 415 GC/pL (Figure 4). Additional samples of target DNA at concentrations ranging from 1500 pM to 0 pM were anaylzed by both the PSTrace and the malaria detection app (Table S1). This evaluation revealed that of the 7 samples tested with the app, only the 1 pM sample was within a 20% agreement with the chronoamperometric reading. Additionally, samples with high concetrations of target returned values below the limit of quantitation, while the negative control returned a value above the limit of quantitation. These results indicate a data processing error within the software itself, as the chronoamperometry plots yielded expected values in the correct range. Future improvements to the software will be implemented to decrease the error seen in the reported results.

4. Conclusion

During this study, we developed a portable, inexpensive Malaria parasite detection technology that pairs with a mobile phone application to deliver results in a point of care setting. Our novel assay utilizes enzyme free SPAAC-mediated ligation to detect target DNA down to picomolar sensitivity (LOD= 100 pM). Our device, known as MalariaSENS™, consists of a portable potentiostat and automated assay. The EmStat Pro chronoamperometry platform was used to validate the electrochemical detection of the target DNA in human whole blood samples that had been spiked with cultured *Plasmodium* parasites. The mobile phone application we developed to deliver results demonstrated its ability to successful connect via wireless Bluetooth connection with the EmStat Go potentiostat. The MalariaSENS™ device will report a positive or negative test result for *P. falciparum* as well as the number of parasites per microliter of blood. We plan on further refinements to the application's algorithm to improve the accuracy of test results. This study presents a strong foundation for the further development of an electrochemical detection device that will be a critical health resource for those in Malaria affected regions as well as low-resource settings. MalariaSENS™ offers rapid, sensitive, and cost-effective malaria diagnostics, advancing healthcare accessibility in resource-limited regions with mobile integration.

Author contributions

D.D., A.A.A and M.R conceived the study and D.D. designed the experiments. M.R. helped D.D. with the bioinformatics, primers design and primer modification. D.D. performed the assay optimization, sensitivity, specificity, and detection experiments in blood samples. M.S. and A.A.A. intellectually contributed to the scientific discussion and data interpretation. D.D., M.S., A.A.A. and M.R wrote the manuscript.

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Competing financial interests

The authors have no conflict of interest.

References

Ariey, F., Witkowski, B., Amaratunga, C., Beghain, J., Langlois, A. C., Khim, N., . . . Menard, D. (2014). A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature, 505(7481), 50-55. doi:10.1038/nature12876

Gilles, H., & Adetokunbo, L. (2002). Short Textbook of Public Health, Medicine for the Tropics (4 ed.). London: CRC Press.

Haldar, K., Bhattacharjee, S., & Safeukui, I. (2018). Drug resistance in *Plasmodium*. Nat Rev Microbiol, 16(3), 156-170. doi:10.1038/nrmicro.2017.161

Holding, P. A., & Snow, R. W. (2001). Impact of *Plasmodium falciparum* malaria on performance and learning: review of the evidence. Am J Trop Med Hyg, 64(1-2 Suppl), 68-75. doi:10.4269/ajtmh.2001.64.68

Imwong, M., Nguyen, T. N., Tripura, R., Peto, T. J., Lee, S. J., Lwin, K. M., . . . Nosten, F. (2015). The epidemiology of subclinical malaria infections in South-East Asia: findings from cross-sectional surveys in Thailand-Myanmar border areas, Cambodia, and Vietnam. Malar J, 14, 381. doi:10.1186/s12936-015-0906-x

Imwong, M., Stepniewska, K., Tripura, R., Peto, T. J., Lwin, K. M., Vihokhern, B., . . . White, N. J. (2016). Numerical Distributions of Parasite Densities During Asymptomatic Malaria. J Infect Dis, 213(8), 1322-1329. doi:10.1093/infdis/jiv596

Imwong, M., Suwannasin, K., Kunasol, C., Sutawong, K., Mayxay, M., Rekol, H., . . . Dondorp, A. M. (2017). The spread of artemisinin-resistant *Plasmodium falciparum* in the Greater Mekong subregion: a molecular epidemiology observational study. Lancet Infect Dis, 17(5), 491-497. doi:10.1016/S1473-3099(17)30048-8

Joanny, F., Lohr, S. J., Engleitner, T., Lell, B., & Mordmuller, B. (2014). Limit of blank and limit of detection of *Plasmodium falciparum* thick blood smear microscopy in a routine setting in Central Africa. Malar J, 13, 234. doi:10.1186/1475-2875-13-234

Kihara, M., Carter, J. A., & Newton, C. R. (2006). The effect of *Plasmodium falciparum* on cognition: a systematic review. Trop Med Int Health, 11(4), 386-397. doi:10.1111/j.1365-3156.2006.01579.x

Maitland, K. (2016). Severe Malaria in African Children - The Need for Continuing Investment. N Engl J Med, 375(25), 2416-2417. doi:10.1056/NEJMp1613528

Noedl, H., Se, Y., Schaecher, K., Smith, B. L., Socheat, D., Fukuda, M. M., & Artemisinin Resistance in Cambodia 1 Study, C. (2008). Evidence of artemisinin-resistant malaria in western Cambodia. N Engl J Med, 359(24), 2619-2620. doi:10.1056/NEJMc0805011

Ouattara, A. F., Raso, G., Edi, C. V., Utzinger, J., Tanner, M., Dagnogo, M., & Koudou, B. G. (2011). Malaria knowledge and long-lasting insecticidal net use in rural communities of central Cote d'Ivoire. Malar J, 10, 288. doi:10.1186/1475-2875-10-288

Rossi, G., De Smet, M., Khim, N., Kindermans, J. M., & Menard, D. (2017). Emergence of *Plasmodium falciparum* triple mutant in Cambodia. Lancet Infect Dis, 17(12), 1233. doi:10.1016/S1473-3099(17)30635-7

Wu, L., van den Hoogen, L. L., Slater, H., Walker, P. G., Ghani, A. C., Drakeley, C. J., & Okell, L. C. (2015). Comparison of diagnostics for the detection of asymptomatic *Plasmodium falciparum* infections to inform control and elimination strategies. Nature, 528(7580), S86-93. doi:10.1038/nature16039

(WHO), W. H. O. (2015a). Guidelines for the treatment of marlaria. 3. Retrieved from http://apps.who.int/iris/bitstream/10665/162441/1/9789241549127_eng.pdf

(WHO), W. H. O. (2015b). World Malaria Report.

(WHO), W. H. O. (2016). Malaria: Diagnostic testing. Retrieved from http://www.who.int/malaria/areas/diagnosis/en/