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Detection Of Plasmodium Falciparum Histidine Rich Protein Ii (Pfhrp2) In Saliva of Malaria Patients: Confirmatory Analysis

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Abstract:

Purpose: *The study aimed to improve malaria diagnostic capacity by detecting the presence of Plasmodium falciparum histidine-rich protein 2 (PfHRP2) in the saliva of malaria patients. The specific objectives were to determine the presence of PfHRP2 in saliva and to evaluate the effectiveness of saliva as a non-invasive diagnostic tool for malaria.*

Material/methods: *A mixed-methods approach was employed, combining both qualitative and quantitative methodologies. The study utilized descriptive and comparative research designs. It was conducted at Jaramogi Oginga Odinga Teaching and Referral Hospital in Kisumu County, with sample analysis carried out at the Bill and Melinda Gates Malaria Laboratory, University of Eastern Africa, Baraton. Purposive sampling was used to select 96 patients suspected of having malaria. Data were collected through ELISA and microscopy tests. Statistical analysis was conducted using SPSS Version 22, including Chi-square testing at a 95% confidence level ($p > 0.05$) to compare diagnostic outcomes.*

Findings: *ELISA detection of PfHRP2 in saliva indicated that 75% of the respondents tested positive, while 25% tested negative. The comparison between ELISA and microscopy showed an agreement with a sensitivity of 88.5%, specificity of 100%, and an overall diagnostic accuracy of 94%.*

Conclusion*: The study concludes that PfHRP2 can be reliably detected in saliva, and that ELISA demonstrates high sensitivity, specificity, and diagnostic accuracy when compared to microscopy. These results suggest saliva-based ELISA testing is a promising non-invasive alternative for malaria diagnosis.*

Value: *This study contributes to ongoing efforts to develop non-invasive, accurate, and field-appropriate diagnostic tools for malaria. It supports the potential integration of saliva-based diagnostics into malaria surveillance and treatment protocols, particularly in resource-limited settings.*

Keywords*: Plasmodium Falciparum, Rich Protein, Pfhrp2, Malaria, Patients*

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1. Introduction

Malaria is one of the most significant infectious diseases globally, causing substantial mortality and morbidity, particularly in tropical regions. Early and accurate diagnosis is crucial for effective treatment and to prevent the overuse of anti-malarial drugs, which can promote resistance (Baker et al., 2010). In 2009 alone, 225 million malaria cases were recorded, leading to 781,000 deaths across 106 endemic countries (WHO, 2010). While clinical algorithms are commonly used for diagnosis, their accuracy is limited due to symptom overlap with other tropical diseases (Baker et al., 2010). Giemsa-stained thick and thin blood smear microscopy, introduced in 1904, remains the gold standard for malaria diagnosis, offering both parasite quantification and species identification (Kifude et al., 2008).

In resource-limited settings where microscopy is not feasible, rapid diagnostic tests (RDTs) serve as a qualitative alternative by detecting parasite antigens from small blood samples (Martin et al., 2009). These tests offer a fast and practical solution for uncomplicated malaria cases (Bendezu et al., 2010). However, blood-based diagnostics are invasive, require trained personnel, and may face compliance issues, especially among children or communities with cultural objections to blood collection (Mharakurwa et al., 2006; Fung et al., 2012). To address these challenges, researchers have explored non-invasive alternatives such as oral fluid, which is safer, less invasive, and easier to collect by moderately trained personnel (Kifude et al., 2008).

Biomarkers for malaria, including PfHRP2, have been detected in saliva, showing potential for diagnosis through immunoassays. Studies have shown correlations between salivary and plasma anti-malarial IgG, and the presence of PfHRP2 in whole saliva was detected at varying sensitivities using ELISA and RDT formats (Wilson et al., 2008; Gbotosho et al., 2010). These findings suggest saliva could be a valuable medium for malaria diagnostics, especially for large-scale or longitudinal surveillance where non-invasive and repeatable sampling is essential (Baker et al., 2010). As countries transition from malaria control to elimination, diagnostic needs are shifting to identify asymptomatic infections and low-density parasitemia, requiring sensitive and practical tools (Harris et al., 2010).

In Kenya, malaria is a leading cause of outpatient visits and hospital admissions, accounting for up to 50% of all outpatient cases and 20% of all admissions (MOH, 2001). It causes an estimated 170 million lost workdays annually and is responsible for 20% of deaths in children under five (MOH, 2006). While public awareness of malaria transmission is high, service provider knowledge, especially concerning malaria in pregnancy, remains low. Regions around Lake Victoria and coastal Kenya experience year-round transmission due to favorable climatic conditions (MOH, 2006). Reliable, accessible diagnostics that are sensitive, affordable, and easy to use are critical in these areas. Although microscopy remains the gold standard, its limitations underscore the need for alternative methods such as saliva-based PfHRP2 detection using ELISA, which offers accurate, scalable, and minimally invasive diagnostics (Kifude et al., 2008). This innovation holds promise for strengthening malaria diagnosis and management, especially in remote or underserved areas. Thus, the study detected the presence of *Pf*HRP2 in saliva of malaria patients and determined the effectiveness of saliva as a diagnostic tool for malaria.

2. Review of Related Literature and Studies

Malaria remains a significant public health challenge, particularly in the developing world, where it causes between 1.5 and 2.7 million deaths annually (Mendiratta et al., 2006; WHO, 2010). The burden is most severe in tropical and subtropical regions, where health infrastructure and control programs are often inadequate (Verma et al., 2013). Plasmodium falciparum is responsible for the majority of malaria-related deaths, especially among children in Africa (Buppan et al., 2010). Despite the implementation of effective interventions like artemisinin-based combination therapies, drug resistance, increased transmission, and human migration continue to undermine malaria control efforts globally (Ambrosino et al., 2010).

Traditional presumptive treatment, often based solely on the presence of fever, has led to the widespread over-prescription of anti-malarial drugs, even when patients do not have malaria parasites (Shillcutt et al., 2008). This practice contributes not only to wastage of drugs but also to the under-treatment of other febrile illnesses, such as bacterial infections. The two main diagnostic approaches—clinical diagnosis and microscopy—each have limitations. Clinical diagnosis lacks specificity due to overlapping symptoms with other diseases, while microscopy is resource-intensive and requires skilled personnel (Baker et al., 2010; Bendezu et al., 2010).

Rapid Diagnostic Test for Malaria

Rapid diagnostic tests (RDTs) offer an effective alternative for malaria diagnosis, especially in low-resource settings where microscopy is not feasible. These tests detect specific parasite antigens, including histidine-rich protein 2 *(HRP2*), Plasmodium lactate dehydrogenase (pLDH), and aldolase, using lateral flow immunochromatography (Gamboa et al., 2010; Mayor et al., 2012). RDTs have demonstrated high sensitivity and specificity in detecting Plasmodium falciparum, particularly when parasite density exceeds 500 parasites/μL (Baker et al., 2010). Their simplicity, speed, and ease of use make them ideal for rural or emergency settings.

Nevertheless*, HRP2*-based RDTs have limitations, including the persistence of antigenemia after treatment, which can lead to false positives and unnecessary treatment (Endeshaw et al., 2012). Additionally, antigenic variability and environmental conditions may affect the performance of RDTs in different geographical regions (Mariette et al., 2008). Despite these concerns, over 150 RDTs are currently available, with *PfHRP2* being the most common target due to its stability and abundance (Hendriksen et al., 2011; Rubach et al., 2012).

Histidine Rich Protein II

Histidine-rich protein II (*HRP2*) is a water-soluble protein specific to P. falciparum and is synthesized throughout its asexual life cycle. It is localized in the infected erythrocyte cytoplasm and membrane, as well as circulating in the bloodstream (Desakorn et al., 1997; Mockenhaupt et al., 2006). HRP2 has a high content of histidine, alanine, and aspartic acid, and it serves as an effective biomarker due to its secretion and accumulation during infection (Howard et al., 1986). It is also involved in the detoxification of free haem, a byproduct of haemoglobin degradation, by facilitating its conversion into haemozoin (Papalexis et al., 2001).

Due to its consistent expression and stability, HRP2 is an excellent target for rapid diagnostic tests (Beadle et al., 1994). The concentration of HRP2 in blood correlates with parasite density, making it a reliable marker for diagnosing active infection. Quantitative studies have reported HRP2 levels ranging from 1.012 fg per parasite in culture to over 1 μg/mL in plasma (Parra et al., 1991; Kifude et al., 2008). These properties make HRP2 one of the most studied and utilized antigens in malaria diagnostics.

The Function of Histidine Rich Protein II

HRP2 plays a crucial role in malaria pathophysiology, particularly in haem detoxification. It has been shown to bind haem and facilitate its polymerization into haemozoin, thereby preventing the toxic effects of free haem on the parasite (Pandey et al., 2001; Sullivan et al., 1996). Studies have also indicated that HRP2 may interact with antimalarial drugs like chloroquine by competing for haem binding, which could influence drug efficacy (Lynn et al., 1999). HRP2's involvement in haem transport and polymerization within the digestive vacuole underscores its importance in parasite survival.

Beyond detoxification, HRP2 may assist in immune evasion by helping the parasite avoid detection and clearance in the spleen (Lopez et al., 2000). It also contributes to cytoadherence and rosetting of infected red blood cells, phenomena associated with severe malaria complications like cerebral malaria (Magowan et al., 2000; Oh et al., 2000). These multifaceted roles further validate HRP2 as a key target for both diagnostics and therapeutic intervention.

Applications of Histidine Rich Protein II

The main application of HRP2 is in malaria diagnostics, particularly through RDTs and ELISA-based assays that detect circulating HRP2 in blood samples (Beadle et al., 1994; Gaye et al., 1998). Dipstick tests such as ParaSight-F and ICT rely on HRP2 detection and have shown high sensitivity and specificity in endemic and non-endemic regions (Shiff et al., 1993; Dietze et al., 1995). These tests are easy to use, require minimal training, and provide rapid results, making them suitable for use in remote areas (Cavallo et al., 1997; Kumar et al., 1996).

Despite their advantages, HRP2-based tests have limitations. Antigen persistence after treatment can lead to prolonged test positivity, complicating monitoring and evaluation of treatment efficacy (Maxyay et al., 2001; Tjitra et al., 2001). The qualitative nature of these tests also limits their usefulness in determining disease severity or guiding clinical decisions in highly endemic areas where semi-immunity is common. Nevertheless, their widespread availability has significantly enhanced malaria screening and early treatment (Jenilek et al., 2003).

Saliva and Malaria Diagnosis

The use of saliva as a diagnostic medium offers a non-invasive and safer alternative to blood, particularly in communities with cultural taboos against blood collection or where needle use poses risks (Kifude et al., 2008). Saliva collection is easier, less painful, and suitable for remote and low-resource settings. Technological advances, such as analyte stabilization and transcriptomic analysis, have improved the sensitivity and specificity of salivary diagnostics (Fung et al., 2012; Biswas et al., 2005).

Studies have demonstrated the presence of malaria antigens like PfHRP2 in the saliva of infected individuals, suggesting its potential for malaria diagnosis (Howard et al., 1986; Purtapontip et al., 2012). Saliva contains trace amounts of parasite proteins, which can be detected using custom ELISA assays. This method enables the development of saliva-based diagnostic kits that could revolutionize malaria detection, especially among children and in areas lacking laboratory infrastructure. However, further research is needed to optimize analyte detection and establish standardized protocols for saliva-based testing (Gbotosho et al., 2010).

3. Material and Methods

Study Design and Setting

This study adopted both qualitative and quantitative research approaches using descriptive and comparative research designs. It was conducted at Jaramogi Oginga Odinga Teaching and Referral Hospital in Kisumu County, a malaria-endemic region influenced by its warm climate and proximity to Lake Victoria. Kisumu's environmental conditions, high humidity and significant rainfall—make it a suitable setting for malaria studies.

Sampling Procedure and Participants:

Purposive sampling was employed to select febrile patients suspected of having malaria. Those testing positive through microscopy were enrolled as cases, and those testing negative served as controls. Demographic data were collected using a structured yes/no Likert-scale questionnaire developed by the researcher.

Blood Sample Collection and Microscopy

Venous blood samples (4 ml) were collected using standard phlebotomy protocols. Thick blood smears were prepared, stained with Giemsa, and examined under oil immersion to estimate parasitemia levels. The parasite density was calculated by averaging the number of infected cells observed across several fields.

Saliva Collection and ELISA Testing

Saliva samples were collected following WHO guidelines, centrifuged, treated with protease inhibitors, and stored at –20 °C. The PfHRP2 antigen in saliva was detected using a sandwich ELISA method. After incubation and multiple washing steps, TMB substrate was used to develop color, and absorbance was read using a spectrophotometer at 450 nm.

Data Analysis

Data collected from both microscopy and ELISA tests were analyzed using SPSS version 22. Descriptive statistics summarized the findings, while Chi-square tests assessed the significance of differences between blood-based and saliva-based malaria diagnoses.

4. Findings and Discussion

This section presents analysis and findings of the study as set out in the research objective and research methodology. To achieve the objective of this study the respondents were highly encouraged and persuaded to respond to the statements in the questionnaire objectively to ensure validity and reliability. The study sought to collect data from 138 respondents, a total of 99 respondents constituting 94% of the respondents' rates. The response rate was adequate for analysis and reporting. Out of the 99 samples collected 96 samples were analyzed by ELISA technique because of the nature of the research instrument used which accommodated only 96 samples.

Sample characteristics

The The demographic profile of respondents was analyzed to provide context on their capacity to inform the study and to identify any gaps that could affect the achievement of the research objectives. The sample included both genders almost equally, with 52.5% male and 48.5% female. Age-wise, a majority (67%) were between 15 and 30 years, while 32.3% were aged 5 to 15 years, indicating a youthful population. In terms of education, most respondents (61.6%) had attained secondary education, while 22.2% had tertiary education and 16.2% had only primary education, showing a fair distribution across educational levels. Occupationally, the majority of respondents (64.6%) were farmers, followed by 17.2% civil servants and 6% businesspersons. This reflects the dominant economic activity in the study area and suggests that the research captured a broad representation of the local population. These demographic insights help contextualize the responses and enhance the interpretation of findings in relation to malaria diagnosis and health-seeking behavior.

Table 1: *Sample characteristics*

		Frequency	Percent
Age	0-15	32	32.3
	15 and above	67	67.7
	Total	99	100
Gender	Male	52	52.5
	Female	47	48.5
	Total	99	100
Level of education	Primary level	16	16.2
	Secondary level	61	61.6
	Tertiary level	22	22.2
	Total	99	100
Occupation	Farmer	64	64.6
	Civil servant	17	17.2
	Business person	*6*	*6.1*
	Total	**99**	**100**

Source: Research Data (2016)

Malaria Patients Characteristics

The summarized statistics from Table 2 reveal key behavioral and preventive trends related to malaria awareness and management among the respondents. Only 12.1% recognized symptoms within two days, while the majority (45.5%) became aware after one week, and 28.3% after two weeks, with 14.1% realizing the symptoms after more than two weeks. This indicates limited early detection, possibly due to low awareness or misattribution of symptoms. About one-third (33%) of the respondents practiced self-medication, primarily due to poor access to healthcare or financial constraints, while the majority (67%) did not. In terms of malaria prevention, 66% reported using insecticide-treated nets, though only 7.1% treated them every three months and 38.4% annually. Alarmingly, 54.5% never treated their nets, which is likely to reduce their effectiveness. Lastly, regarding reinfection, 62.6% had experienced malaria within the last month, 13.1% within two weeks, 15.2% in the last two months, and 9.1% over two months ago. These findings underscore the frequency of malaria recurrence, likely due to inconsistent preventive measures and suboptimal treatment adherence.

Table 2: *Malaria Patients Characteristics*

		Frequency	Percent
Length of the disease symptoms	Two days	12	12.1
	One week	45	45.5
	Two weeks	28	28.3
	More than two weeks	14	14.1
	Total	99	100
respondents on self-medication	Yes	33	33
	No	66	67
Use of insecticide treated nets	Yes	65	66
	No	34	34
Regularity of treating the nets	Every three months	7	7.1
	Every year	38	38.4
	Never	54	54.5
	Total	99	100
Malaria history	Yes	39	39
	No	60	61

Time period since time of the last infection	Two Weeks	13	13.1
	One Month	62	62.6
	Two Months	15	15.2
	More Than Two Months	9	9.1
	Total	99	100

Presence of PfHRP2 in Saliva of Malaria Patients

The first objective of the study was to detect Presence of PfHRP2 in Saliva of Malaria Patients. Table 3 presents the number of patients with PfHRP2 and those without by using negative and positive signs. The study revealed that 75% of the 96 patients tested positive for the presence of PfHRP2 in saliva, while the remaining 25% tested negative. These findings demonstrate a promising detection rate for saliva-based malaria diagnosis. This aligns with a comparative study conducted in Ghana, where PfHRP2 levels were analyzed in plasma and saliva samples of children. In that study, 53% of malaria-positive children showed PfHRP2 presence in plasma, and 43% in saliva, with both sample types showing 100% specificity and no false positives (Wilson et al., 2008). These results highlight the potential of saliva-based PfHRP2 detection as a non-invasive, reliable, and cost-effective alternative to traditional diagnostic methods.

Table 3: *Presence of PfHRP2 in Saliva of Malaria Patients*

	Frequency	**Percent**
Positive	72	75
Negative	24	25
Total	**96**	**100**

Malaria Diagnosis by Microscopy

Table 4 presents malaria diagnosis by microscopy. The figures indicate that 53 per cent tested negative for malaria test by microscopy, 14 per cent tested positive for low levels of malaria parasites by microscopy, 13 per cent tested positive for moderate level parasites and 21 per cent of them tested positive for higher levels of malaria parasites. This suggests that a positive test for malaria parasites by microscopy depends on the concentration levels of the malarial parasites.

Table 4: *Malaria Diagnosis Using Blood Smears (Microscopy)*

	Frequency	Percent
No mps	51	53
Mps +	13	14
Mps ++	12	13
Mps +++	20	21
Total	96	100

Source: Research data (2016)

Effectiveness of Saliva as a Diagnostic Tool for Malaria

The study second objective was to determine effectiveness of saliva as diagnostic tool for malaria. This was investigated by comparing the number of patients with positive or negative malaria test by microscopy and positive or negative test on detection of presence of PfHRP2 in Saliva of Malaria Patients. The findings are presented in table 5. The study compared the negative test results with positive test results for both microscopy and ELISA (PfHRP2) in Saliva this study compares to the research done by Fung et al., 2012 where eight patients with microscopically confirmed P. falciparum malaria tested positive for PfHRP2 in saliva. All negative-control samples tested negative for salivary Pf HRP2. On a binary-decision basis, the ELISA agreed with microscopy with 100 % sensitivity and 100 % specificity.

Table 5: *Cross Tabulation of Positive or Negative by microscopy Test and Positive or Negative (ELISA) PfHRP2 in Saliva of Malaria Patients*

PfHRP2(ELISA)				
		Positive	**Negative**	**Total**
Microscopy_	Positive	44	0	44
	Negative	6	46	52
	Total	50	46	96

Source: Research data (2016)

The study compared the negative test result with positive test result for both microscopy and ELISA in Saliva of Malaria Patients where microscopy had 44 positives and 52 negatives while Elisa had 50 positives and 46 negatives. The statistical difference of malaria diagnosis between Elisa and Microscopy was tested using chi square Test at 95% confidence level (0.05) level of significance. The findings showed there was no significant statistical difference of malaria diagnosis between Elisa with saliva as a diagnostic specimen and Microscopy with blood as a diagnostic specimen (P>0.05).

Table 6: *Chi-Square Test*

		Microscopy				
		Positive	Negative	Total	Chi-square	Sig.
ELISA	Positive	44	6	50	.316[a]	.574
	Negative	0	46	46		
	Total	44	52	96		

Source: Research data (2016)

Diagnostic Sensitivity and Specificity

Measures of Accuracy for Clinical Diagnosis Sensitivity, specificity, positive predictive value and negative predictive value are all measures of accuracy for clinical testing (Wolf et al., 1998a). Sensitivity is the proportion of those individuals who have the disease as is determined by the pathology report that were correctly given the clinical diagnosis of having the disease, i.e. that is, P (positive clinical diagnosis | positive pathological report. Specificity is the proportion of those patients who are pathologically ascertained to be disease-free and are correctly given the clinical diagnosis of being disease-free, i.e. that is, P (negative clinical diagnosis | negative pathological report) (Wolf et al., 1998b). The values of false negative, false positive, true negative true positive are shown in table 7. Thereafter the calculations are shown in equation 1-5.

The study demonstrated a high diagnostic performance for detecting PfHRP2 in whole saliva,

achieving a specificity of 100% and a sensitivity of 88.5%, surpassing the sensitivity of 43% reported by Wilson et al. (2008) using ELISA. Comparatively, Gbotosho et al. (2010) reported lower sensitivities of 77.9% in whole saliva and 48.4% in saliva supernatant using rapid diagnostic tests (RDTs). These comparisons underscore the potential of saliva-based diagnostics while highlighting the continued need for improved sensitivity in saliva tests to match blood-based detection accuracy.

Further supporting the viability of this approach, Fung et al. (2012) achieved perfect sensitivity and specificity (100%) in saliva-based malaria detection. Abba et al. (2011), in a systematic review, reported mean sensitivity and specificity for PfHRP2-based RDTs at 95.0% and 95.2%, respectively, in African contexts. However, false positives remain a concern, particularly due to prolonged antigen persistence post-treatment (Kyabayinze et al., 2011) and cross-reactivity with infections like Schistosoma mekongi (Leshem et al., 2011), underscoring the importance of improving diagnostic precision.

Table 7: *Diagnostic Sensitivity and Specificity*

	Disease	**No Disease**
Positive Test	True positive (44)	False positive (6)
Negative Test	False negative (0)	True negative (46)
Sensitivity and Specificity results		
Sensitivity	100%	
Specificity	88.50%	
Positive Predictive Value	100%	
Negative Predictive Value	100%	
Accuracy	94%	

5. Conclusion

Quantitative studies have measured the levels of Pf HRP2 in blood and established its diagnostic significance. Parra et al (1991) first identified Pf HRP2 in the plasma of infected individuals. Other studies reported average levels of Pf HRP2 in the range of 1.012 fg per parasite and 8.53 fg per infected RBC in culture medium, and 0.57 to 1.11 μg/mL in plasma. Generally, Pf HRP2 is present at higher levels in whole blood than plasma and is released in larger quantities than pLDH. The studies by Desakorn et al. (2005) and Kifude et al. (2008) also reported a relationship between parasite density and plasma levels of parasite antigens.

The study was able detect Pf HRP2 in the saliva of individuals with P. falciparum malaria. Some of the samples that tested negative by microscopy were found to be positive by ELISA test for Pf HRP2 in Saliva. Thus, it will be useful to compare the lower limit of detection in saliva with those achieved by microscopy, lateral flow RDTs, and polymerase chain reaction (PCR). Systematic quantitation of other malaria biomarkers in saliva would identify those with the greatest clinical relevance and diagnostic accessibility. For example, human LDH and aldolase are present in the saliva, which suggests that the corresponding Plasmodium proteins could be detected there as well.

Saliva is a promising diagnostic fluid for malaria when protein degradation and matrix effects are mitigated. If the burdens of training and instrumentation can be alleviated with automated, portable and sensitive assays, the use of saliva can enable a cost-effective approach for the screening of large populations to enable eradication programs to shift from passive to active surveillance and case management.

6. Recommendations

The main aim of the study was to improve the diagnostic capacity for malaria by detecting the presence of PfHRP2 in saliva of malaria patients. The detection of PfHRP2 using ELISA provides an accurate method of diagnosis and also allows for analysis of many samples at a time in Kisumu County. In light of the aforementioned findings, the following recommendations are made:

Saliva is not yet a reliable alternative in the diagnosis of Plasmodium falciparum, but is still a potential alternative and as such, the development of a test kit that is highly sensitive to detect lower levels of the antigen present in saliva and standardization of the sample collection and processing will be a more appropriate approach to malaria diagnostic and in epidemiological surveys. Saliva is a good diagnostic tool for malaria diagnosis as it augments malaria microscopy and ensures prompt diagnosis, and timely treatment; thereby reducing the morbidity and mortality associated with malaria.

The author further recommends that the collection of oral fluid should be detailed because this can significantly affect the composition of the sample. For example, gingival cervicular fluid differs markedly from saliva, which can differ yet depending on whether a specific gland was targeted and whether the collection was stimulated or resting. Where possible, fresh saliva should be used and kept on ice after centrifugation. If analysis is to be done at a later date, the samples should be refrigerated and stabilized with appropriate inhibitors.

Future work should also be able to measure the concentration gradient of biomarkers between blood and saliva with relationship to parasite density.

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