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Saliva And Urine As Diagnostic Specimens For Detection Of Malaria – Review Article

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Abstract
Blood remains the standard for diagnosing malaria, whether microscopic, nucleic acid or biomarker to identify Plasmodium present in the patient. However, given the risk of blood collection, repeated samples taken from unsanitary areas may be useful for malaria testing in resource-limited areas. Although current methods using saliva or urine are not as sensitive and specific as using blood, the potential of these two samples should not be underestimated and efforts have been made to develop a specific method to test for Plasmodium in these two samples. The saliva and feces of people infected with malaria contain trace amounts of Plasmodium DNA and can therefore be used as diagnostic methods. This review describes and summarizes the sensitivity and specificity achieved with different detection methods when these samples are used in the diagnosis of malaria .
Keywords*: Plasmodium; malaria diagnosis; saliva; urine ;Nucleic acid based test ; RDTs*

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I. Introduction

The best diagnostic technique to eradicate malaria would be one that is quick, easy to use, affordable, sensitive, accurate, and non-invasive.The malaria diagnostic techniques that are currently available comprise using microscopy, rapid diagnostic tests (RDT), and polymerase chain reaction (PCR) to identify malaria parasites or parasite proteins in blood.[1]

The gold standard for diagnosis is the microscopic inspection of thick blood films stained with Giemsa. However, even skilled microscopists frequently miss infected patients with extremely low parasitaemia [2,3], since they usually detect only 50–100 parasites or more/microlitre of peripheral blood [4, 5]. Residents of places with low malaria transmission rates are reservoirs for the disease and carry submicroscopic parasitemia [6, 7]. RDTs identify proteins specific to malaria parasites, such as HRP-2 (histidine-rich protein 2) in plasma. RDTs are appropriate for field settings and are simple to use. Despite the fact that multi-survey analyses demonstrate a significant correlation between malaria prevalence measured by microscopy and by RD [8],compared to microscopic examination, RDTs identify more infections [2, 4, 9].However, after parasite clearance, HRP-2-based RDTs may continue to produce false-positive results for up to one month [3, 9,10,11]. PCR can detect as little as 1-4 parasites/µl of blood, demonstrating the remarkable sensitivity of molecular approaches [12, 13, 14, 15]. Blood sample requirements are a common issue throughout microscopy, RDT, and nested PCR (nPCR) for malaria diagnosis, despite variations in their methods and capabilities. Blood collection carries some risk. Blood collection must be performed by trained personnel and can be problematic for some people, especially children, people with trypanophobia, and people who have received blood transfusions [16]. Blood letting may be associated with injury or side effects such as pain and hematoma, but sometimes serious complications such as accidental infection can occur, especially in resource-poor settings [17,18]. Additionally, at-risk patients may lose compliance with the recommended frequency [19], which may hinder continuous monitoring of malaria diagnosis or treatment due to the small number of participants in biological studies [20].Therefore, non-invasive and rapid malaria testing devices that use other body fluids are ideal for healthcare, especially in peripheral areas.

Both urine and saliva are good physical products to test for biomarker or diagnostic purposes. No special equipment is required to collect either type of sample, and therefore a simple, inexpensive, quantitative and aggregative method is possible that can be performed outside the hospital by people with little training, including patients. Additionally, there is no need for blood cell lysis, which could limit the presence and detecti on of antigen [21].

Since urine is the ultrafiltrate of blood, many low molecular weight plasma or serum proteins can be detected in urine, and these proteins are suitable for investigating not only the course of kidney disease but also the underlying disease [22]. Serum proteins are filtered by the glomeruli according to their size and charge, and

many proteins, including albumin, light immunoglobulin, and transferrin, are reabsorbed in adjacent renal tubules [23]) .A total of proteins are excreted in healthy individuals in amounts less than 150 mg per day, and more than 6000 total proteins have been measured in normal human urine to date [24]. Studies have shown that 30% of urine proteins come from plasma proteins [25], Another recent study showed that 2940 (47.7%) of the gene products detected in urine were compared with those in plasma (81.1%) [24]. This study also showed that proteins from 44 tissues were found in urine, brain, stomach and colon. These are the tissues with the highest number of highly expressed proteins in urine, both protein and mRNA [24].In non urinary tract infections, viral antigens found in the blood or antibodies to pathogens such as Helicobacter pylori [26] *dengue virus* [27])and *Streptococcus pneumoniae* [28] can also be detected in urine. Antigens and antibodies are also filtered from the blood into the urine. Additionally, as a result of the breakdown of DNA from dying human cells and microbes, some extracellular nucleic acids in plasma and blood pass through the kidneys and are excreted in the urine as transrenal DNA.These DNA fragments arecurrently known targets for polymerase chain reaction (PCR)based detection of microbial diseases such as *Mycobacterium tuberculosis* [29] and cancers such as breast cancer [30]. Therefore, the presence of plasma or blood proteins and transrenal DNA is of great value for the diagnosis of urinary tract infections or the detection and monitoring of infections. Collecting saliva is much easier than collecting urine because it can be collected anytime, anywhere without needing a toilet or a place to urinate. Fluid from the blood enters the salivary ducts, where it is converted from isotonic fluid to hypotonic fluid, forming the basis of saliva [31].The normal protein content of saliva is 0.72. 4 mg/ml, but there were significant differences in protein content according to collection time , gender, age and disease status [32]. Approximately 2290 proteins characterizing the protein structure of whole saliva have been reported in various studies. Of the 2698 proteins identified in plasma, 27% were salivary proteins. Instead of 60:80% of the total weight being dominated by immunoglobulins and albumin as observed in plasma, the top 20 most abundant proteins were found to account for only 40% of salivary proteins [33] .This could improve the identification of organisms from 60% of the remaining proteins [34] .The functions of salivary secretion include lubrication, anti microbial process, protection of muscle integrity and digestion [33]. Studies have shown that 7% of all salivary proteins are identified as immunoglobulins, and 58% of these are found in plasma; this suggests that these immunoglobulins migrate from plasma to saliva [33].The apparent linear distribution of immunoglobulins (isotypes, subtypes) between plasma and saliva indicates that antibodies in saliva can be detected with a linear concentration in plasma. This explains the basis for the development of saliva antibody tests for SARS-CoV-2, HIV[, and HBV [.35.36.37].

On the other hand, saliva containing markers such as antigens, DNA, and 16S RNA sequences are frequently used as targets for saliva-based diagnostic tools, even though viral diseases are not detected in saliva . Despite the few shared profiles of proteins, the gene ontology distribution of salivary and plasma proteins is quite similar [33]. This also highlights the importance of saliva for clinical use in disease diagnosis and health assessment.

II. Nucleic Acid Based Test

Nucleic acidbased diagnostic methods for malaria have been developed and used by researchers due to their high accuracy, sensitivity, and specificity in detecting Plasmodium in patient samples.The advent of highly sensitive techniques such as quantitative PCR and digital PCR allows the detection of Plasmodium at very low parasitemia or gene copy numbers.The ease of designing primers to amplify modified regions and specific sequences provides specificity in detecting Plasmodium in other organisms or parasites, as well as in identifying different Plasmodium species present in samples.The only nucleic acid based method developed, tested, and reported to date for saliva and urine samples is the use of nested PCR and isothermal amplification (LAMP), which amplify the most common genes of Plasmodium, such as 18S ribosomal RNA. 18SrRNA).[38]

Nested PCR

Nested PCR (nPCR) has been shown to be more sensitive and superior to microscopy in the detection of circulating Plasmodium, especially Plasmodium-type pathogens. Identification of Plasmodium falciparum (Pf) in saliva and urine using PCR was first reported in 2006 by Mharakurwa and his team [39] ; here they were able to identify merozoitetype protein 2 (MSP2) and dihydrofolate redhydrase (DHFR). Pf to strengthen. Saliva and urine site with geometric mean parasitemia of,775 unique parasites/µl. However, specific emotions and details are not mentioned by the authors. Another study among Gambians using nPCR but amplifying the 18S rRNA region of Pf achieved a sensitivity and specificity of 32% and 98% based on urine and a sensitivity and specificity of 73% and 97%, respectively, for saliva samples. The sample is compared with the results obtained from microscopic examination [40]. The sensitivity of Pf detection increased to 82% for saliva samples with a parasite density of 0001000 parasites/µl; this is probably the level of parasitemia seen in most malaria patients in the Gambia and other malaria regions. The use of this method was extended by Buppan and colleagues to identify other Plasmodium species such as Plasmodium vivax (Pv) [41] .Compared with microscopy results, 18S rRNA nPCR of saliva samples had a sensitivity of 74.1% and 84% for the detection of Pf and Pv and a sensitivity of 44.4%

and 34% for the detection of Pf and Pv. , in urine. The specificity of nPCR for both saliva and urine was 100% for Pf and Pv in cases compared to nPCR from blood. The geometric mean parasite density in this study was 2761 parasites/ml for Pf and 1248 parasites/ml for Pv.18S rRNA nPCR was subsequently reported in two studies showing that Pv and Pf in saliva and urine could provide a >90% increase in sensitivity compared to microscopic results [42.43] .The increase was reported in a recent study using the same method for the detection of Pf in saliva samples; by which sensitivity and specificity were 95% and 93%, respectively, when referring to microscopy results, while corresponding values were 82% and 99% when blood nPCR was taken as reference standard [44].

Nested PCR was later developed to increase the sensitivity and specificity of Plasmodium detection. Instead of the extra 18S rRNA, which is found in four to eight copies in each parasite nucleus, the mitochondrial cytochrome b gene is found in the small genome (mtDNA) of up to 6 kb with a copy number of 30 to 100 per parasite.] In addition to the high copy number of the mitochondrial cytochrome b gene, which can increase the diagnosis sensitivity of Plasmodium, the low level of variation of this gene sequence can also distinguish Plasmodium species. In a previous study on the mitochondrial cytochrome b gene, the overall detection limit of the nPCR assay was 10 copies/μL (150 copies) for five Plasmodium species [45].

Compared with blood nPCR, the sensitivity of detecting Pf and Pv in saliva was 74.2% and 79.2%, which was higher than 18S rRNA nPCR (Pf: 52.8%; Pv: 61.0%). Similarly, higher sensitivity was also observed in mitochondrial cytochrome b gene nPCR of urine samples; sensitivity was 55.1% for Pf and 53.3% for Pv detection compared to 18S rRNA (Pf: 25.8%; Pv: 14.3%). The specificity of this assay in detecting Pf and Pv from saliva and urine samples ranged from 97.5% to 100%, comparable to that of 18S rRNA nPCR. For Pv, the positive detection rate of mitochondrial cytochrome b gene nPCR of saliva reached 100% even when parasite density was <1000 parasites/μl, whereas the study yielded a positive rate of only 75% at this level of parasite density in urineand remained virtually unchanged despite increased parasitemia.For Pf, positive saliva detection rates of >80% were obtained even for parasitemia <1000 parasites/μl, while positive urine values showed a strong correlation with parasite density, although the optimal detection rate was 75%. This PCR assay also demonstrated the ability to detect Pf and Pv in saliva and urine with submicroscopic parasitemia. This PCR test was performed on patients with good performance in Southern Iran;saliva and urine samples each had a specificity of 97% and a sensitivity of 91% and 70%, respectively.[46] However, microscopic results were used as standard measurements in the studies. Additionally, no correlation was observed between parasite density and positive saliva and urine nPCR results.Surprisingly, one study used Pf Kelch 13 (PfK13), Pf dihydrofolate reductase (Pfdhfr) and Pf chloroquine transporter (Pfcrt) genes as nPCR genes to detect Plasmodial DNA in saliva and urine [47]. The study showed a 46%, 64%, and 5% positive rate for PfK13, Pfdhfr, and Pfcrt genes in saliva samples compared to blood samples, while only PfK13 and Pfdhfr protein could be found in urine, per 45% and 38% sensitivity.The specificity of the results was generally ≤50%. The copy number is the justification for using these antimalarials as the purpose is not stated by the authors. However, these genes may be useful in identifying patients infected with Plasmodium species that have antimalarial genes and may help better treat malaria. This study also determined that saliva was the best blood sample formolecular diagnostic tests for malaria. However, in a previous study, a high sensitivity of 91% with a specificity of 50% could be achieved in detecting the Pfcrt gene from Pf in saliva samples [48].

Loop-Mediated Isothermal Amplification

Loop mediated isothermal amplification (LAMP) is a nucleic acid amplification method that amplifies cells in isothermal conditions at 65°C, allowing real time visualization of developing cells with the naked eye through color changes. In addition to not requiring an expensive device such as a thermal cycler, LAMP is a simple, economical and rapid method that can be completed in 30 minutes with a sensitivity 10-100 times higher than conventional PCR and 500-1000 times higher than PCR. antigen. detection [42.49]. Considering all the benefits that LAMP can provide, it has become a popular method for diagnosing various infectious diseases, including malaria, especially in endemic regions with poor infrastructure and limited technical resources.The first LAMP test for Pv in saliva was performed by Singh et al,In this study, the 18S rRNA of Pv was targeted, and compared with the microscopic results, the sensitivity and specificity were 76.3% and 94.1%, respectively. However, another study aiming to determine Pf and Pv in saliva and urine using 18S rRNA LAMP found a significant reduction in overall sensitivity compared to microscopy of up to 48.5% for saliva and 30% for urine , but the overall specificity of the two Plasmodium species detection was 100% for both saliva and urine samples [42]. The detection rate of 18S rRNA LAMP showed a strong correlation with parasite density in blood; the maximum detection rate was 64.7% for saliva and 52.9% for urine. Later, Modak and colleagues [49] attempted to improve LAMP targeting the mitochondrial cytochrome oxidase subunit 1 gene using saliva without nucleic acid purification. Saliva from sample collectors was subjected to LAMP and identified as Pf positive. However, saliva .samples from patients were not tested because diagnostic performance was evaluated only in Pf-infected normal saliva at a parasite density of ~4255 parasites/μl.

Other Nucleic Acid-Based Methods

In addition to nPCR and LAMP, recent studies have attempted to improve the detection of Pf and Pv in saliva using digital droplet PCR (ddPCR) , with concurrent evaluation using quantitative PCR. In addition to nPCR and LAMP, recent studies have attempted to improve the detection of Pf and Pv in saliva using digital droplet PCR (ddPCR) and one-time detection using PCR (qPCR) [(50)] . DdPCR targeting Pf346 and Pvr47 was able to amplify 76% of Pf and 57% of Pv in saliva samples positive for ddPCR in blood; The overall sensitivity of ddPCR in saliva was 73% . However, although the sensitivity of ddPCR was slightly lower than qPCR, the specificity of ddPCR (100%) was higher than qPCR (55%). Additionally, ddPCR was found to be more effective than qPCR in detecting mixed diseases. The detection rate of Pvr47 and Pfr364 in blood by DdPCR was 0. 1-0. Pf and 0 for 9 parasites/µL. 9- 2. 7 parasites/µl for Pv.Identification of Plasmodium in saliva and urine using real time nucleic acid sequencing (QTNASBA) has also been attempted. Realtime QTNASBA is a method that includes RNA extraction, RNA target amplification and internal control, and end product detection by electrochemiluminescence (ECL) [(51)] or molecular fluorescence [(52)]. Real-time QTNASBA targeting 18S rRNA was first adapted from qualitative NASBA for Plasmodium identification by Schoone et al. [(51)], giving a sensitivity of 10–50 parasites/ml with assays. Schneider et al. However, real-time QT-NASBA is preferred over real-time QT-PCR due to the advantages of realtime QTNASBA such as fast turnaround time, easy extraction of RNA, and activation of human blood. Realtime QTNASBA has also shown a detection limit of up to 0.02 gametocytes/µl in dried spots on filter papers when amplifying gametocyte-specific Pfs25-mRNA [(53)].

Regarding saliva and urine, the 18S rRNA detected in real-time QTNASBA in saliva was moderate (80%), but the target could be added only to two-thirds of the urine (66.7%) [(54)].On the other hand, the detection rate of Psf16 mRNA from Pf gametocytes was low for both saliva and urine, and surprisingly the sensitivity of urine (20%) was slightly higher than that of saliva (13.3%). As for Pfs25 mRNA, none of the saliva and urine samples showed positive results in the QT-NASBA test, probably due to the higher detection rate of 1710 compared to 143 RNA copies of Pfs16 mRNA. It has been hypothesized that the low detection of Psf16 and Psf25 mRNA may be due to the fact that mature gametocyte mRNA is not expressed at all, the sensitivity of the method is too low, or the mRNA is highly degraded in the body [(54)].

Conclusion

In general, the sensitivity for detecting Plasmodium in saliva samples (70-80%) is higher than in urine samples (30-50%), making saliva suitable as a non-invasive sample for the detection of Plasmodium DNA. This can be explained based on evidence of a 600fold reduction in the amount of parasite DNA in saliva samples compared to that in the peripheral blood of infected patients, while urine contained the lowest number of parasite DNA.DNA was ~2500. - lower than those in peripheral blood . Additionally, while parasitemia was observed to be excreted from the blood into saliva, no correlation was found between parasitemia and parasite DNA in urine [(40)].

Of the nucleic acid methods tested to date, nPCR provided the best sensitivity and 18S rRNA was still the preferred gene, but high copy number mitochondrial genes such as cytochrome b and cox3 were also found to provide high performance in Pf detection. However, the role of mitochondrial genes in Plasmodium LAMP recognition has not yet been determined. Most nPCR methods were able to detect Plasmodial DNA with parasitaemia of 0001000 parasites/µl in saliva, regardless of the gene target used. nPCR is also better at detecting infections caused by Plasmodium species [(41)] compared to microscopy. Interestingly, some studies have demonstrated the ability of PCR to detect Plasmodial DNA.However, some difficulties must be overcome when using saliva and urine samples to detect plasmodiumbased nucleic acids. In addition to gene selection, DNA extraction and primer design are important to determine the efficiency and effectiveness of PCR in detecting low plasmodial DNA in saliva and urine. It has been shown that extraction from a commercial kit provides higher amplicon yield than the Chelex method, and as parasite density increases, amplicon yield increases by% [(39)]. Primers that produce longer amplicons have also been found to produce lower amplicon yields [(39)]

The sensitivity of the PCR test may also depend on the volume and storage conditions of the sample. Due to the low level of parasite DNA found in saliva and urine, the amount of sample collected can greatly affect the yield of parasite DNA extracted and therefore increase the sensitivity of the PCR test, especially for single assay [(40)] Ethanolpreserved field samples showed superior performance in detecting Plasmodium by nPCR compared to samples stored on ice without preservation; This suggests that antimicrobial agents may play an important role in preventing microbial contamination of field samples, which could prevent the extraction of Plasmodial DNA from being disturbed[(41)] and allows samples to be stored at room temperature without significant degradation of the DNA in the samples [(44)]. However, the storage time of saliva stored at -20°C or -80°C was found to have no significant effect on qPCR and ddPCR [(50)].

Another issue that should not be overlooked is the long-term approach to nucleic acid-based malaria diagnosis, including DNA extraction and PCR, especially in vitro PCR. Although LAMP can overcome the longterm variability of conventional or implanted PCR, methods using saliva or urine have not yet been

developed. Although one-step DNA extraction has been tested on saliva samples, it is still in its infancy and has not yet been tested on saliva samples from patients .Therefore, more efforts are needed to to improve the preparation time of DNA from saliva or urine samples. [49].

Antigen-Based Diagnostic Methods

Rapid diagnostic tests (RDTs), often referred to as antigen-based diagnostic techniques, are widely employed in conjunction with microscopic analysis of blood smears to identify Plasmodium antigens in blood or plasma. Because of its speed, ease of use, and affordability, this method enables laboratory or clinical staff, regardless of expertise level, to diagnose malaria at the point of care in endemic rural settings and places with little laboratory resources. This technology is somewhat akin to nucleic-acid diagnostic techniques like PCR, although it is far more sensitive and selective than microscopy [55].

RDTs are rarely utilized or assessed for the detection of Plasmodium species in non-invasive specimens, such as saliva and urine, despite being widely employed on blood samples. Saliva-based rapid diagnostic tests (RDTs) have proven to be an efficient means of self-diagnosing the novel coronavirus SARS-CoV-2 that emerged in 2019 [56], the human immunodeficiency virus (and the measles, mumps, rubella, and hepatitis [57] ·This method typically detects the parasite antigen in the blood of infected people using an immuno-chromatographic device impregnated with monoclonal antibodies against Plasmodium species [58], Numerous studies have tried to assess the effectiveness of malaria RDTs on saliva and urine samples because of the many advantages of using non-invasive specimens for malaria diagnosis. Plasmodium falciparum lactate dehydrogenase (pLDH) and Plasmodium falciparum histidine-rich protein 2 (PfHRP2) are the most often found target antigens by malaria RDTs. Additionally, some studies looked for different antigens or antibodies that would be better suited for detecting Plasmodium in urine and saliva samples, or they developed novel tools or methods for diagnosing malaria in urine and saliva.[38]

Pf HRP2 and pLDH

Among the bodily fluids that contain HRP2 are saliva and urine [38] .The use of malaria RDTs to identify malaria in these types of bodily fluids has gained attention due to a number of findings. Using a dipstick-style test called the ParaSightR-F, Genton et al. [59] conducted the first investigation to identify PfHRP2 in urine samples. On comparison to the results of blood PCR and microscopy, the sensitivity and specificity of Pf detection were approximately 25% and 80%, respectively. The urine-based malaria test kit (UMT), developed by Fyodor Biotechnologies Baltimore, USA, uses recombinant monoclonal antibody to detect highly repetitive cognate polyhistidine-rich protein 2 (HRP2) and fragments that are in the urine of febrile patients. This was done because blood-based malaria RDTs did not yield satisfactory results when used for malaria detection in urine .agreements of 80–90% but specificities of 80% -95% were achieved .UMT can detect parasites as low as 120 parasites/µl, and the optimal detection rate is highly correlated with parasite density [58,60]. However, the study was able to achieve sensitivity and specificity compared to UMT, i.e. 86.67% and 94.12%, when using a bloodbased test kit (BinaxNOW Malaria Test kit, Inverness Medical, Europe) to detect Pf in urine·[61]. On the other hand, use of two other blood-based RDT CareStartTM Malaria (Accessbio, USA) and Global Devices Malaria (USA) kits for PfHRP-2 detection gave a low sensitivity of 67–80% with >95% specificity [62] . Unsurprisingly, UMT does not always seem to promise the sensitivity and specificity noted above; because a recent study achieved only 55.4% sensitivity and 47.5% specificity compared to microscopy results [63] Unlike urine, no commercially available malaria RDTs have yet been developed specifically for saliva purposes. All studies evaluated the use of saliva for the diagnosis of malaria using a blood-based RDT. When targeting PfHRP2, the malaria ELISA kit detected Pf in saliva with only 43% sensitivity and 100% specificity compared to microscopy [21]. Another study instead identified another known protein from Pf, pLDH, and found a higher sensitivity of 77.6% and specificity of 100% compared to microscopy [48]. A recent study to test for malaria through saliva and urine tests used an RDT that can detect PfHRP2 and pLDH antigens. Compared with blood test results, the sensitivity in detecting PfHRP-2/pLDH from blood-contaminated urine and saliva was 35.2% and 57%, but decreased to 7.6% and 13.3% in purified blood samples .Specificity for all sample types was 100%. The same study also showed a detection limit of parasites of 63,150 parasites/µl for urine and 57,335 parasites/µl for saliva, as well as a significant correlation between detectionrates and parasite density [64].

However, another study where parasitemia was much lower, ranging from 105 to 7200 parasites/µl, used the same RDT but a higher sensitivity of 70% could be achieved for urine and saliva compared to microscopic results and blood PCR.The study found a specificity of only 77.1% to 93%, rather than 100%. Detection of 1% in urine andPf in saliva. [65]

Other Antigens/Antibodies Approach

In addition to direct antigen detection, antibodies to Plasmodium antigens have also been tested against malaria. Estevez et al. [66]evaluated the use of ELISA, which measures IgG antibodies against Pf antigens,

merozoite-type protein-1 (MSP-119), and apigene membrane antigen (AMA-1) as an alternative method to detect Pf in saliva samples. They also compared Pf activity in saliva collected using conventional methods and two commercially available kits. For both antigens, antibody levels in plasma correlated significantly with those in saliva collected using commercial kits, AMA -1 (r2 range). 0. 93 to 0.89, p<0. 001) and MSP-119 (r2 range 0.93 to 0.75, p < 0.001). 001), a weak correlation was observed with saliva collected using the normal method (r range 0.64 to 0.63, p < 0.01). Using plasma as the standard, the sensitivity and specificity of the salivary antibody AMA-1 ranged between 64-77%,, and 91-100%, while values for MSP-119 ranged between 47-67% and 90-97%. . With a wide variety of sampling methods. Commercial sampling equipment did not appear to provide better performance in the detection of Pf in saliva samples compared to conventional sampling methods in this study.As mentioned above, there is no saliva-based malaria vaccine available on the market to date. Tao et al. [67] reported the development of a prototype saliva-based tissue specific immunoassay (LFIA) that identifies Plasmodiumtype protein 17 (PSSP17) in Pf gametocytes because subclinically infected individuals carry high levels of gametocytes. As a reservoir of malariacausing parasites transmitted by mosquitoes.PSSP17 was selected from 35 Pf proteins detected in infected parasite carriers using tandem liquid chromatography-mass spectrometry (LC-MS/MS) and confirmed using LC-multiplex sequencing (LC-MRM) and qPCR analysis. When microscopy comparing the gametocyte-specific transcript pfs25 in blood and blood 18S rRNA PCR, qPCR results, PSSP17 LFIA was able to detect gametocytes with sensitivity of 100%, 92%, and 91% with a detection limit of 0.7 gametocytes/µL. In addition, according to microscopy results, trophozoites can also be detected with the test with 92% sensitivity. In fact, in addition to the RDTs mentioned above, there are many other studies trying to identify antigens or specific antibodies in saliva or urine to identify Plasmodium species, or to develop new methods to identify Plasmodium species in both body fluids. One study used a very different method, namely Rolling-Circle-Enhanced-Enzyme-Activity-Detection (REEAD), which involves DNA amplification to detect the activity of a biomarker to detect Plasmodium species in blood [68]. The system is based on an isothermal cleavage/ligation event on single DNA, specifically initiated by the Plasmodium enzyme topoisomerase I, following mixing of circular DNA by primer immobilization and amplification in a Rolling Circle Amplification (RCA) response. A circular DNA template is converted into a rolling circle product (RCP) of ~103 tandem repeats that can be easily visualized at the single-molecule level .This system, together with the droplet microfluidic Lab-on-a-Chip, enables the detection of all infectious, specific and quantitative malaria-causing Plasmodium species in a single drop of unprocessed blood with a minimal number of parasites/µL [69] .To achieve the highest performance of SOMA, other studies have tested this method for saliva identification of Plasmodium species with some modifications to meet point-of-care (POC) testing requirements; i) a non-invasive system, including replacement of the pump-driven microfluidic device pump and ii) microscopic measurements and colorimetric measurements based on horseradish peroxidase (HRP) activity .[70]) Therefore, pTopI could be detected in the saliva of all 35 malaria patients, while Plasmodium infection could be detected in saliva samples even in patients with parasitemia of less than 2 parasites/µL in the corresponding blood. The results of this study showed that the READ-based pTopI assay is promising for use in the POC diagnosis of malaria using saliva as a testing tool. One of the newest technologies being developed for the detection of different target chemical or biological analytes is the biosensor. Many biosensors have been created for use in healthcare to test oxygen and glucose levels, as well as to identify tumors and viruses. Diagnostic applications are increasingly using impedimetric biosensors because of their high sensitivity, affordability, and ability to be miniaturized.[71] .Different kinds of PfHRP2 immunosensors were developed based on direct or sandwich immunoassays for the diagnosis of malaria; the detection limit was as low as 10 fg/mL in spiked plasma and 18 fg/mL in spiked whole blood .[72].Soraya et al. [71] created the first biosensor to identify PfHRP2 in saliva samples. They did this by creating an interdigitated electrode (IDE) sensor that could identify PfHRP2 linked to anti-PfHRP2 monoclonal antibodies (MAbs) deposited on the sensor surface in an impedimetric and label-free manner. In 8 of the 11 examined samples, they showed a promising feasibility of PfHRP2 detection in saliva, with a detection limit of 25 pg/mL. [71]

III. Conclusions

As a result, poor performance of Pf antigen in saliva and urine has been observed in currently marketed malaria RDTs. RDTs designed to detect Pf in blood samples may not be suitable for use in urine and saliva.While UMTs appear to provide specific and specific measurement of urinary Pf, studies using specific RDTs to detect salivary Pf have also demonstrated the potential of saliva as an alternative method choice for malaria testing.The main problem of RDTs is their limitations in detecting diseases with low parasitemia [65]. The minimum detection limit for field tests was set at 200 parasites/µl blood[72]. In a study describing the correlation between antigen detection and parasite density, a malaria infected blood group with total parasitemia of 200/µL required at least 4 ng/mL PfHRP2 to achieve 95% efficiency, whereas ≥45 ng/mL pLDH was required is required for at least 90% of positive results [73]. Additionally, current screening for malaria elimination needs to be improved to detect the number of asymptomatic carriers. For this, the measurement limit of the PfHRP2 diagnostic test must be 1-2 times less than the current RDT .In addition, false positive results may be reported due to the persistence of the target

antigen even after successful treatment and the presence of rheumatism and schistosomiasis in the patient. The presence of gametocytes and cross-reactivity of the RDT with other antigens can also lead to false positives [(61).].For Plasmodium detection in urine and saliva, possible factors leading to low sensitivity of RDTs may include low levels of parasitemia, low antigen production of the parasite due to mutation or gene deletion, blank or timing of sample collection, presence of antibodies against the parasite. , like that. The HRP2 antigen is characterized by antibodies (monoclonal and/or polyclonal) injected into RDT kits.Detection ability can be obtained from free morning urine rather than urine collected later. Parasite monitoring can also reduce antigenemia and ultrafiltration of antigens in urine [(61)] ·Additionally, proteins excreted in the urine may be reduced or proteolytic and difficult to detect by RDTs designed to detect antigens in the blood [(39)]. The effect of sample storage method on RDT performance could not be determined because none of thestudies compared it, although studies did show high sensitivity in detecting Pf using whole saliva (77. 9%) compared to the supernatant of spun saliva (48.4%) stored at 4°C for 24 h before centrifugation .[(42)] Although the use of commercially available saliva collection devices has been described, saliva collected from these devices did not provide better detection of Pf than urine or saliva stored on ice or at −20°C for reasons or was processed immediately. for RDT after collection due to the ease of use of RT.Most studies have not produced satisfactory results due to the limitations of commercially available kits intended to detect higher levels of PfHRP2 in whole blood or plasma rather than saliva and urine. Therefore, the development of a kit or test that is sensitive and specific enough to detect low levels of antigen in saliva and urine may be an appropriate measure in malaria diagnosis and epidemiological studies.

Some studies reported that UMT provided higher sensitivity than blood-specific RDT measured in urine, with a cut-off of 120 parasites/μL and a sensitivity of 50% for ≤200 parasites/μL [(58)]. However, this study found that the specificity of UMT obtained from this study was still below 90%. The poor performance and false positive results may be due to the above factors. Therefore, rather than detecting HRP2 with current UMT, the discovery and detection of other Plasmodium antigens which are more specific and abundant in urine may be necessary to overcome the abovementioned issues and develop a more sensitive UMT.Overall, there is still no single method that can meet all requirements for malaria detection in a resource-limited environment. These parameters; These are non-invasiveness, low cost, speed, simplicity, ease of use, no need for sample preparation, minimal involvement of expert personnel, combination of high sensitivity and specificity, and low detection limit. In fact, the low sensitivity and specificity of this diagnostic method may be due to the lack of microscopic examination during the period when microscopy was considered the standard of care. Inaccuracy of microscopic examination may be due to inability to identify malaria species at low parasitemia, inexperienced microscopists, and malaria-like materials [(74)]

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