Tropical Biomedicine 35(3): 781–795 (2018)

Clinical enzymes as possible immunological biomarkers in the diagnosis of malaria, human African and American trypanosomiasis

Raphael Enrique Tiongco[1,9*], Noemi Anne Paragas[2,9], Micah Angela Salunga[3,9], Mark F F Padua[4,9], Dea Ponciano[5,9], Marri Vyzielle Pinpin[5,9], Mark Raymund Nava[6,9], Maileen Ragasa[7,9] and Maria Ruth Pineda-Cortel[3,8,9]
[1]Department of Medical Technology, College of Allied Medical Professions, Angeles University Foundation, Angeles City, Philippines
[2]Institute of Clinical Laboratory Sciences, Silliman University, Dumaguete, Negros Oriental, Philippines
[3]Department of Medical Technology, Faculty of Pharmacy, University of Santo Tomas, Manila City, Philippines
[4]Department of Medical Technology, Institute of Arts and Sciences, Far Eastern University, Manila City, Philippines
[5]Department of Clinical Pathology, University of Santo Tomas Hospital, Manila City, Philippines
[6]College of Medical Laboratory Science, Our Lady of Guadalupe Colleges, Inc., Mandaluyong City, Philippines
[7]Department of Pathology, Bataan General Hospital, Balanga City, Bataan, Philippines
[8]Research Center for the Natural and Applied Sciences, University of Santo Tomas, Manila City, Philippines
[9]The Graduate School, University of Santo Tomas, Manila City, Philippines
[*]Corresponding author e-mail: tiongco.raphael@auf.edu.ph
Received 18 February 2018; received in revised form 14 May 2018; accepted 5 July 2018

Abstract. Peripheral blood smear microscopy still remains the gold standard for diagnosing malaria and trypanosomiasis. Microscopy is a labor-intensive process and requires great amount of skill to accomplish. Even though cheap and easy to perform, it still has several limitations. This hinders the microscopist in identifying protozoan structure or differentiating species from one another. Considering these factors in the performance of microscopic examination, it is crucial to identify new strategies for parasite identification and species differentiation. Innovations in clinical enzymology, immunodiagnostics, and molecular technology would be of help in resolving the problem. This study mainly focused on the possible role of clinical enzymes in malaria and trypanosomiasis diagnosis. Enzymes play a vital role in parasite physiology and metabolism. They enable the parasite to survive inside the living host by initiating different metabolic cycles. These enzymes can either be expressed on the surface of the protozoa or excreted in the extracellular environment. Lactate dehydrogenase, aldolase, and glutamate dehydrogenase were the significant enzymes associated with pathogenic *Plasmodium* spp. Other malarial enzymes were also identified but further validation is still required to establish their use as diagnostic biomarkers. Whereas, the enzyme matrix metalloproteinase was identified as significant for diagnosing and differentiating *Trypanosoma* spp. Analysis of these enzymes can be used as alternative means for microscopy in parasite identification and differentiation. Application of these enzymes as immunologic markers in various diagnostic test kits should be further evaluated.

INTRODUCTION

Microscopic evaluation of peripheral blood smear is considered as the cornerstone of diagnostic testing for blood and tissue protozoan infections, namely *Plasmodium* and *Trypanosoma*. Although effective, it has low sensitivity due to the difficulty in identifying different morphological stages of the parasite. Factors such as parasite size and density, specimen consistency and volume, collection and transport of specimen should be taken into consideration to enhance the diagnostic performance of microscopy. Moreover, microscopy is a labor-intensive process and requires great amount of expertise. Currently, we have a shortage of skilled laboratory scientist capable of identifying the eggs and adult forms of the parasite in wet and dry smears of clinical specimens (Bergquist *et al.*, 2009; Boatin *et al.*, 2012; L'Ollivier and Piarroux, 2013; Tangpukdee *et al.*, 2009). Considering these factors, it is vital to identify new strategies to improve parasite identification and species differentiation such as the use of clinical enzymology, immunodiagnostics, and molecular technology. In this review article, we focused on the use of enzymes as possible immunological biomarkers in the detection and differentiation of *Plasmodium* and *Trypanosoma* species. The study aimed to identify alternative techniques to routine blood film microscopy that is cost effective, more sensitive, readily available, and easily performed.

ARTICLE SELECTION AND SEARCH CRITERIA

References for this review were based on searches in PubMed, ScienceDirect and Google Scholar. Search strategy included combinations of the following key words: "enzymes", "enzymology", "malaria", "*Plasmodium*", "*Trypanosoma*", and "trypanosomiasis". All resulting articles were initially screened by checking the abstract. Studies included are those that focused on the use of enzymes as biomarkers in the diagnosis of either malaria or trypanosomiasis. Articles that passed the inclusion criteria were further screened by checking the references for relevant citations. Overall, a total of 13, 995 (published between 1988 and 2017) studies were screened by the researchers. Out of the total number of studies screened, only 34 were considered relevant for this review article.

EPIDEMIOLOGY OF MALARIA

Malaria infection is considered a threat to public health in approximately 91 countries. It is caused by five species of *Plasmodium* parasites, namely: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*. The parasite is usually transmitted through the bite of a female *Anopheles* mosquito. Higher rates of transmission are usually observed in countries with warm climates such as those near the equator. In these countries, the disease is transmitted all-year-round. In countries with cooler climates, the disease transmission is less intense and more seasonal. The highest rates of malaria transmission are seen in South Africa and Papua New Guinea. According to the World Health Organization (WHO) World Malaria Report in 2016, there were a total of 212 million individuals affected by malaria and 429, 000 have died due to the disease. In 2017, wherein children aged 5 and below were susceptible to the infection, more than 70% of all deaths due to malaria occur in this age group. Aside from the African region, Southeast Asia, Latin America, and the Middle-East are also at risk ("CDC," 2017, "WHO," 2017).

All species of *Plasmodium* are found to cause severe infections; however, infections caused by *P. falciparum* are classified to be the deadliest and exhibit the most cases of drug resistance. Among the human parasite species, *P. vivax* is the most prevalent species in temperate climates and the causative agent of relapsing benign tertian malaria (Gething *et al.*, 2012; Gething *et al.*, 2011). In sub-Saharan Africa, *P. falciparum* is the most prevalent while *P. ovale* primary infects the western areas of sub-Saharan Africa. Although *P. knowlesi* normally infects Southeast Asian macaques, human infections also have been on the rise. In fact, it is responsible for approximately 50% of malaria cases in Malaysia. *P. knowlesi* infection is difficult to diagnose since different stages of *P. knowlesi* closely resemble *P. malariae*. But, unlike the usually benign infections with *P. malariae*, *P. knowlesi* infections can be rapidly fatal (Gething *et al.*, 2012; Gething *et al.*, 2011; Mendis *et al.*, 2001).

DIAGNOSIS OF MALARIA

Improper diagnosis and rapidly evolving drug resistant malarial parasites are seen as reasons for the persisting high mortality of malaria in endemic places. WHO recommends that proper diagnosis must be given to all malaria suspected patients before drug administration. This prompted the need to develop fast, economical, and accurate techniques for malaria diagnosis (Jain *et al.*, 2014). Currently, the gold standard for malarial diagnosis is through the examination of a Giemsa-stained thick and thin blood film (Mirdha *et al.*, 1997; Wilson, 2013). However, this method is labor-intensive, time consuming, and requires considerable skill in microscopy. The main disadvantage of microscopic technique would be its low sensitivity, particularly in cases of low parasite density (Erdman and Kain, 2008; Kyabayinze *et al.*, 2010; Payne, 1988). Even though microscopy is less costly and requires inexpensive reagents and equipment, it is important to identify or evaluate new strategies to improve the diagnosis of malaria.

The quantitative buffy coat (QBC) technique was designed to improve microscopic detection of malarial parasites. This new method involves staining of parasite deoxyribonucleic acid with fluorescent dyes (e.g. acridine orange) (Adeoye and Nga, 2007; Chotivanich *et al.*, 2007; Clendennen *et al.*, 1995). The newly developed technique has shown to be more rapid and more sensitive compared to the traditional thick and thin blood film in low parasitaemia, but requires a fluorescent microscope (Ifeorah *et al.*, 2017; Ochola *et al.*, 2006; Salmani *et al.*, 2011; Wongsrichanalai *et al.*, 1992). Therefore, this is not suitable for rural settings with limited resources.

Rapid diagnostic tests (RDT) were recognized by the WHO as simple, quick, and accurate means of diagnosing malaria. Testing using RDTs is inexpensive and is readily available and easy to perform. These tests are able to overcome the deficiencies and shortcomings of the traditional stained blood film examination. Most of RDTs available detect the antigenic enzymes produced by *P. falciparum*. The use of RDTs has been recognized globally and currently used for malarial diagnosis especially in endemic countries (Amexo *et al.*, 2004; Chilton *et al.*, 2006; Desai *et al.*, 2007; Doderer *et al.*, 2007; Endeshaw *et al.*, 2008; Kim *et al.*, 2008; Kyabayinze *et al.*, 2008; Lee *et al.*, 2011; Park *et al.*, 2006; Ratsimbasoa *et al.*, 2008; Wongsrichanalai *et al.*, 2007).

Microscopy, together with RDT, is the recommended method to confirm malarial cases prior to anti-malarial drug administration (Jimenez *et al.*, 2017). Nowadays available RDTs use plasmodial enzymes as the antigen for detection with the exception of histidine-rich protein 2 (HRP2). It follows the same principle, lateral flow immune-chromatography, and comes in various forms such as plastic cassette, dipstick, or card. Antibodies conjugated to colloidal gold particles bind specifically with parasite antigens. While in diagnosing infections caused by *P. falciparum* or by non-*P. falciparum* malaria, antigens common to all species like *Plasmodium* lactate dehydrogenase (pLDH) or aldolase are detected together with HRP2 in some RDTs (Bell and Peeling, 2006). Other plasmodial enzymes that are studied as potential biomarkers are glutamate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase (Krause *et al.*, 2017; Li *et al.*, 2005).

MALARIAL ENZYMOLOGY

A. Lactate Dehydrogenase

pLDH is a key enzyme in the glycolytic pathway of *Plasmodium* species and has isomers specific to the species. This enzyme is secreted in the host's peripheral blood and is detectable within 24 hours of effective malaria treatment (Harani *et al.*, 2006; Oduola *et al.*, 1997). Due to these characteristics, pLDH has become a known reliable marker in detecting the presence of viable *Plasmodium* in the blood and is also widely used for screening in malaria-endemic countries (Lee *et al.*, 2012). However, it must be noted that pLDH is only detectable in the presence of live parasites (Makler and Hinrichs, 1993; Piper *et al.*, 1999).

Because of the lack of a functional Krebs cycle, *Plasmodium* parasites heavily relies on LDH for survival during their intraerythrocytic stages and therefore, the only source of their adenosine triphosphate is through glycolysis together with fermentation. Consequently, an increase in glucose consumption of 30- to 50-fold higher than the host erythrocytes is observed (Chaikuad *et al.*, 2005; Makler and Hinrichs, 1993). pLDH can be differentiated from mammalian LDH in both structure and kinetic features. In terms of structure, pLDH has a five-residue insertion (DKEWN) in their active site loop which activates during catalysis and closes down the active site. This insertion greatly enhances the substrate-specificity of pLDH compared to the human muscle and heart LDH isotopes. When it comes to kinetic feature, all pLDH differ from mammalian LDH by the susceptibility of the later to be inhibited by excess levels of the substrate pyruvate while pLDH exhibits decreased marked substrate inhibition. Also, pLDH has an ability to readily use the synthetic coenzyme 3-acetylpyridine adenine dinucleotide (APAD) as its cofactor (Chaikuad *et al.*, 2005; Makler and Hinrichs, 1993). A study conducted by Brown *et al.* (2004) compared the structure of the pLDH of the four human plasmodial species. They have reported a 90-92% structure similarity of the pLDH from *P. vivax*, *P. malariae* and *P. ovale* to pLDH from *P. falciparum*. However there are significant differences between the *Plasmodium* species when it comes to kinetic properties and sensitivity to inhibitors.

pLDH has been first studied in the 1970s as a biomarker for malaria and had been used to differentiate malarial species. It had also been assayed as a parasite purity indicator. And since pLDH is an effective target for antibody-based malaria diagnosis by numerous researches, easy-to-operate RDTs that follow the principle of lateral flow immunochromatography had been developed. Although light microscopy is the reference method for malaria diagnosis, RDTs have become widely used today since they do not require a microscope or trained microscopist. Proper execution of the procedure provided by the manufacturer is the main requirement for these tests. Further, a diagnosis can be given within a few minutes at point of care (Shoemark *et al.*, 2007). Currently, the most commercially available RDTs are detecting either plasmodial HRP2 (pHRP2) or pLDH (Piper *et al.*, 2011). In a study by Ugah *et al.* (2017), they evaluated three malaria diagnostic methods namely the light microscopy method, molecular method and RDTs. They have reported that microscopy is still a good method for malarial diagnosis since it has a good measure of agreement with the polymerase chain reaction (PCR). They recommended that RDTs with high specificity and sensitivity must be used in combination with microscopy to ensure accuracy of laboratory reports. Another malarial diagnostic approach being studied is the development of antibodies recognizing each species of human *Plasmodium*. Selection of species-specific epitopes is possible since pLDH is not fully conserved across *Plasmodium* species. Different levels of sensitivity of immunochromatographic rapid tests can also result from diverse combinations of monoclonal antibodies against pLDH. These methods present a tractable way to enhance immunochromatographic pLDH tests (Jimenez *et al.*, 2017; Piper *et al.*, 2011).

B. Aldolase

Another major enzyme involved in the glycolytic pathway of *Plasmodium* is aldolase, a homotetrameric protein which catalyses the cleavage of fructose-1,6-bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Each subunit of the enzyme has a molecular weight of approximately 40 kDa. Aldolase can be found in the host's blood when the enzyme is released during infection or in the parasite's cytoplasm in its soluble and active form (Döbeli *et al.*, 1990; Knapp *et al.*, 1990; Srivastava *et al.*, 1990). *Plasmodium* aldolase can be distinguished from human isoenzyme by its possession of some unique nucleotide sequences (Tritten *et al.*, 2009). Both *P. falciparum* and *P. vivax* have only one aldolase isoenzyme and a great proportion of the amino acid sequences are greatly conserved in all *Plasmodium* species (Kim *et al.*, 1998; Lee *et al.*, 2006). In RDTs, aldolase is usually used as a pan-malaria antigen. Since *Plasmodium* aldolase is highly conserved during evolution, it makes the enzyme a target of choice when analysing isolates (Tritten *et al.*, 2009). However, in a study by Bell *et al.* (2005), they have reported that in comparison to HRP2 based RDTs, aldolase and pLDH based RDTs are less sensitive due to the transient presence of the enzymes in the blood. A number of studies have also showed poor sensitivity of aldolase RDTs which called for further studies on the genetic diversity of aldolase. In contrast, a study by Lee *et al.* (2006) on the diversity in *P. falciparum* and *P. vivax* aldolase showed results that aldolase is not a cause of low RDT sensitivity. However, when it comes to detecting *P. vivax* infection, aldolase as a target antigen showed a more reliable diagnosis as reported by Dzakah *et al.* (2014) who assessed the relative performance of four RDTs that emphasized the detection of *P. vivax* antigens. Consequently, researchers have noted that a more sensitive assay for diagnosis of *P. vivax* infection can be developed combining aldolase and pLDH in RDTs.

C. Glutamate Dehydrogenase

Glutamate Dehydrogenase (GDH) plays an important role in the metabolism of carbon and nitrogen. It catalyses the oxidative deamination of L-glutamate to 2-oxoglutarate and ammonia, a NADP-linked reaction. GDHs are assumed to be NADP-dependent when involved in glutamate catabolism while in ammonia fixation, GDH uses NADPH. NADPH production in the *P. falciparum* is mainly the responsibility of NADP-dependent GDH. NADPH serves as the electron source for glutathione reductase and thioredoxin reductase, the parasite's antioxidative enzymes. Consequently, because plasmodia are sensitive to oxidative stress, anti-malarial drug development studies show GDH as a promising target. In addition to this, the host erythrocytes do not contain GDH making this plasmodial enzyme a more desirable target for anti-malarial drug therapy (Wagner *et al.*, 1998; Werner, *et al.*, 2005).

In a purified NADP-specific GDH isolated from *P. falciparum* infected human erythrocytes (Krauth-Siegel *et al.*, 1996), *P. falciparum* GDH (*Pf*GDH) was characterized as a homohexamer with a subunit molecular mass of 49,500 as estimated by SDS/PAGE. A study by Wagner and colleagues in 1998 also described the three-dimensional structure of *Pf*GDH using an X-ray crystallography to a resolution of 2.7 Å. They have stated that the hexameric proteins subunit interfaces are the most prominent differences between plasmodial GDH and human GDH. In addition to this, a unique N-terminal extension can be found in *Pf*GDH which is not seen in other GDH sequence studied (Wagner *et al.*, 1998; Werner *et al.*, 2005). *Pf*GDH also differs from mammalian GDH in a number of aspects namely kinetics, cofactors specificity, substrate, degree of affinity and immunogenicity (Rodríguez-Acosta *et al.*, 1998).

As a favourable malarial biomarker, GDH was also studied for its potential in detecting *P. falciparum* infection. A study was conducted by Li *et al.* (2005) wherein they established a colloidal gold-immunochromatography assay (GICA) in combination with monoclonal antibodies against *Pf*GDH for diagnosis of *P. falciparum* malaria. They had reported that in comparison to routine microscopic examination, GICA had a sensitivity of 86.66% and specificity of 96.43% for *P. falciparum* detection. In another study conducted by de Dominguez and Rodríguez-Acosta (1996), they determined the antigenicity of *Pf*GDH by affinity chromatography isolation and its usefulness as a diagnostic biomarker using enzyme-linked immunoassay (ELISA). Using ELISA, the optical density was significantly higher among malarial patients compared to healthy individuals. Whereas, there was no significant difference in the optical density between the sera of acute malaria patients and the sera of patients with relapse or re-infection. This method was 100% sensitive in diagnosing malaria, but it could not differentiate acute infections from relapse or re-infection. The same authors also conducted another study in 1999, wherein, they used immunoaffinity separation technique with Western Blot analysis to characterize *P. falciparum* antigens present in patient plasma. They had demonstrated in the study that the activity of *Pf*GDH in the plasma could be detected by a technique with no interference from human GDH, making *Pf*GDH an excellent parasite biomarker comparable to lactate dehydrogenase and aldolase (Rodríguez-Acosta *et al.*, 1999).

D. Other Malarial Enzymes

Enzymes included in this section are potential biomarkers for malarial diagnostics but with limited literatures available, such as glyceraldehyde-3-phosphate dehydrogenase (GADPH), thioredoxin peroxidase-1 (TPx-1), dihydrofolate reductase-thymidine synthase (DHFR-TS), hypoxanthine phosphoribosyltransferase (HPRT), phosphoglycerate mutase (PGM), and fructose biphoshate aldolase (FBPA). Further validation is still required to establish the diagnostic use of these enzymes.

GAPDH is another plasmodial glycolytic enzyme that was recently studied as a new malarial diagnostic biomarker. A recombinant *P. falciparum* GAPDH (r*Pf*GAPDH) has been crystallized to determine the three-dimensional structure of the enzyme in a study by Satchell *et al.* (2005). They have reported that the four subunits of the tetrameric enzyme have one molecule of the cofactor NAD+ bound to each. They have also identified the insertion of a dipeptide (-KG-) in the S loop as the major structural feature that differentiates human GAPDH from *Pf*GAPDH (Satchell *et al.*, 2005). It has been shown in previous studies that the amino acid sequences of glycolytic enzymes are highly conserved among *Plasmodium* species. In a study by Krause *et al.* (2017), it was demonstrated that GAPDH can be used in RDTs as a promising alternative to pLDH. Their results had shown the presence of GAPDH in all *Plasmodia* and that it is 80% to 95% conserved amongst the 5 human malaria species. The study had also demonstrated the species-specific characteristic of the enzyme due to the slight variation of the amino acid sequences. Two isotopes specific to *Pf*GAPDH and one common to all mammalian malaria species had been identified by the study. Further research is still being conducted to support plasmodial GAPDH as a new malarial biomarker.

TPx-1 belongs to the family of ubiquitous enzymes called peroxiredoxin, with a molecular weight of approximately 20-30 kDa. TPx-1 is a cytoplasmic enzyme that reduces and detoxifies hydrogen peroxide. Studies showed that *P. falciparum* TPx-1 (*Pf*TPx-1) is highly expressed during the asexual erythrocytic life cycle of the parasite. This enzyme is one of the most expressed in the cytoplasm of *P. falciparum*, accounting for 0.25% to 0.5% of the total cellular protein. Moreover, due to its abundance, consistent expression, and its difference from the human orthologue, this enzyme is a promising target for malarial diagnosis (Gretes *et al.*, 2012; Sue *et al.*, 2005). In a study by Hakimi *et al.* (2015), they developed monoclonal antibodies against *Pf*TPx-1 and incorporated them to different RDTs and tested their role as potential biomarkers. Based on the results, the RDTs were able to detect *Pf*TPx-1 present from in-vitro cell cultures. These findings further suggest that *Pf*TPx-1 is a promising biomarker for *P. falciparum* diagnosis.

DHFR is an enzyme that functions in the folate pathway of *Plasmodium* spp. by catalysing the reduction of dihydrofolate to tetrahydrofolate. This enzyme is unique compared to higher eukaryote homologue because it can form a bi-functional enzyme with thymidine synthase (TS) among protozoa. The DHFR-TS enzyme play an important role in pyrimidine and DNA synthesis in all protozoa, and the production of tetrahydrofolate in plasmodia is highly dependent on the presence of this enzyme. The plasmodial DHFR-TS differ from those of other protozoa due to the existence of two additional sequences in the DHFR domain. Moreover, the sizes of these sequences have a slight variation per *Plasmodium* spp. making this enzyme a potential candidate for species differentiation. Kattenberg *et al.* (2012) studied the potential of this enzyme as new diagnostic marker for malaria. Based on the results of their study, using ELISA, antibodies produced against DHFR-TS (D6 and D15) were able to detect *P. falciparum* crude parasite antigen. These antibodies are comparable to the specificity of commercially available antibodies against HRP2 in detecting *P. falciparum*. Aside from *P. falciparum*, other antibodies produced against DHFR-TS (D7, D15, D16, and D28) were able to detect *P. vivax* antigens from pooled patient samples using ELISA. This suggests that DHFR-TR is a potential biomarker for *P. falciparum* and *P. vivax* differentiation (Kattenberg *et al.*, 2017; Mouatcho and Goldring, 2017).

Recent studies used proteomics in the search for new and effective malarial biomarkers. In a study conducted by Theìzeìnas *et al.* in 2013, they examined the potential of *P. falciparum* hypoxanthine phosphoribosyltransferase (*Pf*HRPT) and other enzymes as a candidate biomarker for acute *P. falciparum* infections using proteomic analysis. Protozoan parasites lack certain enzymes necessary for both DNA and RNA production from small molecules. Instead, they rely on the salvage of hypoxanthine, guanine, and possibly xanthine via phosphoribosyltransferases for both survival and reproduction (Keough *et al.*, 2010). Based on proteomic analysis of blood samples from patients with severe falciparum malaria, mild falciparum malaria, and the control group, hypoxanthine phosphoribosyltransferase, phosphoglycerate mutase (*Pf*PGM), and fructose biphoshate aldolase (*Pf*FBPA), were higher in severe malaria cases compared to the other groups. Based on correlational analysis, they found out that *Pf*FBPA is significantly correlated with parasite density while *Pf*HRPT showed a good correlation with malarial anaemia (Fischer *et al.*, 2013; Mouatcho and Goldring, 2017).

EPIDEMIOLOGY OF TRYPANOSOMIASIS

Human African trypanosomiasis (HAT) epidemics were significant public health problems in the past, but recently, only 7,000 to 10,000 cases are being recorded annually. Human trypanosomiasis is caused by the parasite *Trypanosoma brucei* which is being transmitted by the bite of a carrier tse-tse fly (Genus *Glossina*). There are two subspecies of *T. brucei* that are known to infect man, namely: *T. brucei gambiense* and *T. brucei rhodesiense*. The two subspecies are found in different geographic locations. *T. brucei rhodesiense* is mainly found in areas of Eastern and Southeastern Africa hence the term East African trypanosomiasis. *T. brucei gambiense* on the other hand is predominantly seen in central Africa and in some limited areas of western Africa hence the term West African trypanosomiasis. Before, approximately 60 million individuals were at risk for trypanosomiasis with an estimated 300,000 new infections per year in Africa. But from 1995 to 2014, the rates declined where in 2014 only 3, 796 new cases were reported ("CDC," 2017, "WHO," 2017). Aside from HAT, another form of trypanosomiasis exists, and is known as Chagas disease or American trypanosomiasis. Chagas disease is a potentially life-threatening condition caused by the protozoa *T. cruzi*. Approximately 6 to 7 million people worldwide are infected with this disease, mostly in Latin American countries. The disease is transmitted by the triatomine or kissing bug which is endemic to these areas ("CDC," 2017, "WHO," 2017).

DIAGNOSIS OF TRYPANOSOMIASIS

The routine method for trypanosome diagnosis and differentiation would be the evaluation of a Giemsa-stained blood film.

Although affordable, blood film examination is still not that reliable due to the difficulties in distinguishing the morphological stages of *Trypanosoma*. Other method that can be used for blood examination would be through microscopic examination of the micro-hematocrit buffy coat. This method gives more accurate results with a sensitivity of 68.65%. In this technique, the trypanosomes are being concentrated in the white blood cell zone between the plasma and erythrocytes for easier recovery (Lutumba *et al.*, 2006; Miezan *et al.*, 1994).

Serodiagnostic techniques can also be used in trypanosomiasis diagnosis. ELISA test kits are available in the market for trypanosome antibody detection. This type of method is 97.35% specific and 91.4% sensitivity in trypanosomiasis diagnosis. Even though specific and sensitive, ELISA methods cannot differentiate between subspecies of *T. brucei* complex and cannot differentiate acute from past infections. Other limitations of this technique include the need of specialized equipment and trained staff to perform the test (Elrayah *et al.*, 2007; Hasker *et al.*, 2010; Lejon *et al.*, 2006; Nantulya *et al.*, 1992; Nantulya, 1997). A cheap, quick, and practical serologic test that has been widely used in field diagnosis of HAT is the card agglutination trypanosomiasis test (CATT). This test has high specificity when used on undiluted whole blood, however, the positive predictive value of this test is limited since the test is mainly used on mass screening of populations in which prevalence of HAT is less than 5% (Chappuis *et al.*, 2004).

Molecular techniques, using PCR, can also be used for trypanosome detection in different body fluids. This type of method gives the highest sensitivity and specificity. Most methods used primers that target the 177 bp satellite DNA which permits the detection of members of the Genus *Trypanosoma*. However, this method cannot discriminate the two pathogenic subspecies of *T. brucei* complex. Other drawbacks of this technique include the need for specialized machines that would replicate and detect the presence of the targeted gene and the trained staff to execute the sophisticated test (Deborggraeve *et al.*, 2011; Kabiri *et al.*, 1999; Kanmogne *et al.*, 1996; Penchenier *et al.*, 2000; Radwanska *et al.*, 2002).

HUMAN AFRICAN TRYPANOSOMIASIS ENZYMOLOGY

Various types of proteinases are implicated in ECM degradation, but the major enzymes considered are the matrix metalloproteinases (MMPs). To reach the inner tissues in its host, the parasite *T. brucei* secretes proteases into the ECM, such as the 40kDa neutral metalloproteinase that permits the parasite to move and migrate by degrading collagen, fibronectin, and laminin. The GP63 zinc metalloproteinase, the most important MMPs in the parasite, is a surface enzyme that was first reported in *Leishmania*. This protein is highly conserved among species in terms of homology. This enzyme performs several functions in different stages of the trypanosome life cycle. MMPs are zinc-dependent protein and peptide hydrolases. They are widely involved in metabolism regulation through both extensive protein degradation and selective peptide-bond hydrolysis. MMPs are regulated via modulation of gene expression, compartmentalization, and inhibition by protein inhibitors. Most MMPs are not constitutively transcribed, but are expressed after external induction by cytokines and growth factors. In addition, some MMPs are stored in inflammatory cell granules, which restrict their compass of action (Grandgenett *et al.*, 2007; Löffek *et al.*, 2011; Nagase *et al.*, 2006; Tallant *et al.*, 2010).

The *T. brucei* genome encodes three groups of zinc metalloproteinases, each of which contains ~30% amino acid identity with the major surface protease (MSP, also called GP63) of *Leishmania*. One of these proteases, TbMSP-B, is encoded by four nearly identical, tandem genes transcribed in both bloodstream and procyclic trypanosomes. TbMSP-B is a surface-localized zinc metalloproteinase that is expressed predominantly in differentiating bloodstream form to procyclic form cells and in established procyclic form cells (Grandgenett *et al.*, 2007).

AMERICAN TRYPANOSOMIASIS ENZYMOLOGY

Aside from the possible role of MMPs in HAT diagnosis, they can also be used in the diagnosis of American trypanosomiasis or Chagas Disease. Increased levels of various MMPs such as collagenases, stromelysins, and gelatinases have been associated with inflammatory diseases of connective tissues. Among these collagenases are the MMP-2 and MMP-9, which can be used in the staging and progression of Chagas disease. The former is expressed in all cells including cardiomyocytes and is considered as the most ubiquitous while the latter is expressed in inflammatory cells. MMP-2 and MMP-9 are regulated by tissue inhibitors of MMPs (TIMPs). TIMPs act as key local regulators of activities of MMPs. Aside from identifying the progress of Chagas disease, the two MMPs are upregulated in cardiac tissue during acute phase of *T. cruzi* infection. The upregulation increases the MMP-9 mRNA level as well as the protein content and enzymatic activity. For MMP-2, it degrades specific sarcomeric proteins and its levels can be detected using PCR. The importance of the MMPs as biomarkers in other human studies were already been observed in hypertension, myocardial infarction, and systolic heart failure (Bautista-López *et al.*, 2006; Roberto *et al.*, 2017).

MMP-2 and MMP-9 are also produced by a variety of cells, such as astrocytes, microglia and neurons and they play an important role in neuro-inflammation. As demonstrated in Central Nervous System (CNS) disorders, these two MMPs are involved in Blood-Brain Barrier (BBB) permeability by attacking the extracellular matrix. Human African trypanosomiasis presents different stages and the second stage of the disease indicates elevated levels of MMP-2 and MMP-9 that is also due to the correlation of the number of white blood cells in the cerebrospinal fluid confirming the role in BBB dysfunction (Ngoyi *et al.*, 2011).

CONCLUSION

Based on the review of selected literatures, certain enzymes play a crucial role in blood protozoan physiology and metabolism. Aside from aiding in normal protozoan function and life cycle, these enzymes can also be used as biomarkers for diagnosis. Malarial enzymes emphasized in the study include lactate dehydrogenase, aldolase, and glutamate dehydrogenase. These enzymes showed good results in the diagnosis of malarial infection and at the same time *Plasmodium* species differentiation. Other malarial enzymes were also identified such as glyceraldehyde-3-phosphate dehydrogenase, thioredoxin peroxidase-1, dihydrofolate reductase-thymidine synthase, hypoxanthine phosphoribosyltransferase, phosphoglycerate mutase, and fructose biphoshate aldolase, however, further studies are still required to validate these enzymes as potential diagnostic biomarkers. Whereas, matrix metalloproteinases are the prime enzymes identified in association with African trypanosomiasis. Expression of these enzymes in the surfaces of trypanosomes could be used as antigenic determinants for detecting *Trypanosoma* and differentiating it from *Leishmania*. In general, identification of enzymes mentioned in the study can be used as possible alternative for routine microscopy in the diagnosis and differentiation of *Plasmodium* and *Trypanosoma* species. Possible application of these enzymes as immunologic markers in various diagnostic test kits should be further studied.

REFERENCE

Adeoye, G.O. & Nga, I.C. (2007). Comparison of Quantitative Buffy Coat technique (QBC) with Giemsa-stained thick film (GTF) for diagnosis of malaria. *Parasitology International* **56**: 308-312.

Amexo, M., Tolhurst, R., Barnish, G., Bates, I., McConnico, C.C., Amexo, M. & Thuma, P. (2004). Malaria misdiagnosis: effects on the poor and vulnerable. *The Lancet* **364**: 1896-1898.

Bautista-López, N.L., Morillo, C.A., López-Jaramillo, P., Quiroz, R., Luengas, C. & Silva, S.Y. (2006). Matrix metallo-proteinases 2 and 9 as diagnostic markers in the progression to Chagas cardio-myopathy. *American Heart Journal* **165**: 558-566.

Bell, D. & Peeling, R.W. (2006). Evaluation of rapid diagnostic tests: malaria. *Nature Reviews Microbiology* **4**: 34-40.

Bell, D.R., Wilson, D.W. & Martin, L.B. (2005). False-positive results of a *Plasmodium falciparum* histidine-rich protein 2-detecting malaria rapid diagnostic test due to high sensitivity in a community with fluctuating low parasite density. *American Journal of Tropical Medicine and Hygiene* **73**: 199-203.

Bergquist, R., Johansen, M.V. & Utzinger, J. (2009). Diagnostic dilemmas in helmin-thology: what tools to use and when? *Trends in Parasitology* **25**: 151-156.

Boatin, B.A., Basáñez, M.-G., Prichard, R.K., Awadzi, K., Barakat, R.M., García, H.H. & Lustigman, S. (2012). A Research Agenda for Helminth Diseases of Humans: Towards Control and Elimination. *PLoS Neglected Tropical Diseases* **6**: e1547.

Brown, W.M., Yowell, C.A., Hoard, A., Vander Jagt, T.A., Hunsaker, L.A., Deck, L.M. & Vander Jagt, D.L. (2004). Comparative structural analysis and kinetic properties of lactate dehydrogenases from the four species of human malarial parasites. *Biochemistry* **43**: 6219-6229.

CDC. (2017). Retrieved October 1, 2017, from https://www.cdc.gov/

Chaikuad, A., Fairweather, V., Conners, R., Joseph-Horne, T., Turgut-Balik, D. & Brady, R.L. (2005). Structure of lactate dehydrogenase from *Plasmodium vivax*: Complexes with NADH and APADH. *Biochemistry* **44**: 16221-16228.

Chappuis, F., Stivanello, E., Adams, K., Kidane, S., Pittet, A. & Bovier, P.A. (2004). Card agglutination test for trypanosomiasis (CATT) end-dilution titer and cerebrospinal fluid cell count as predictors of human African trypanosomiasis (*Trypanosoma brucei* gambiense) among serologically suspected individuals in Southern Sudan. *American Journal of Tropical Medicine and Hygiene* **71**: 313-317.

Chilton, D., Malik, A.N.J., Armstrong, M., Kettelhut, M., Parker-Williams, J. & Chiodini, P.L. (2006). Use of rapid diagnostic tests for diagnosis of malaria in the UK. *Journal of Clinical Pathology* **59**: 862-6.

Chotivanich, K., Silamut, K. & Day, N.P.J. (2007). Laboratory diagnosis of malaria infection – A short review of methods. *New Zealand Journal of Medical Laboratory Science*.

Clendennen, T.E., Long, G.W. & Kevin Baird, J. (1995). QBC® and Giemsa-stained thick blood films: Diagnostic per-formance of laboratory technologists. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**: 183-184.

de Dominguez, N. & Rodriguez-Acosta, A. (1996). Glutamate dehydrogenase antigen detection in *Plasmodium falciparum* infections. *The Korean Journal of Parasitology* **34**: 239-246.

Deborggraeve, S., Lejon, V., Ekangu, R.A., Ngoyi, D.M., Pyana, P.P., Ilunga, M. & Büscher, P. (2011). Diagnostic accuracy of PCR in gambiense sleeping sickness diagnosis, staging and post-treatment follow-up: A 2-year longitudinal study. *PLoS Neglected Tropical Diseases* **5**: e972.

Desai, M., ter Kuile, F.O., Nosten, F., McGready, R., Asamoa, K., Brabin, B. & Ayeni, F. (2007). Epidemiology and burden of malaria in pregnancy. *The Lancet Infectious Diseases* **7**: 93-104.

Döbeli, H., Trzeciak, A., Gillessen, D., Matile, H., Srivastava, I.K., Perrin, L.H. & Certa, U. (1990). Expression, purification, biochemical characterization and inhibition of recombinant *Plasmodium falciparum* aldolase. *Molecular and Biochemical Parasitology* **41**: 259-268.

Doderer, C., Heschung, A., Guntz, P., Cazenave, J.-P., Hansmann, Y., Senegas, A. & Candolfi, E. (2007). A new ELISA kit which uses a combination of *Plasmodium falciparum* extract and recombinant *Plasmodium vivax* antigens as an alternative to IFAT for detection of malaria antibodies. *Malaria Journal* **6**: 19.

Dzakah, E.E., Kang, K., Ni, C., Tang, S., Wang, J. & Wang, J. (2014). Comparative performance of aldolase and lactate dehydrogenase rapid diagnostic tests in *Plasmodium vivax* detection. *Malaria Journal* **13**: 272.

Elrayah, I.E., Rhaman, M.A., Karamalla, L.T., Khalil, K.M. & Buscher, P. (2007). Evaluation of serodiagnostic tests for *T.b. gambiense* human African trypanosomiasis in southern Sudan. *Eastern Mediterranean Health Journal* **13**: 1098-1107.

Endeshaw, T., Gebre, T., Ngondi, J., Graves, P.M., Shargie, E.B., Ejigsemahu, Y. & Richards, F.O. (2008). Evaluation of light microscopy and rapid diagnostic test for the detection of malaria under operational field conditions: a household survey in Ethiopia. *Malaria Journal* **7**: 118.

Erdman, L.K. & Kain, K.C. (2008). Molecular diagnostic and surveillance tools for global malaria control. *Travel Medicine and Infectious Disease* **6**: 82-99.

Fischer, R., Digleria, K., Walther, M., Conway, D.J., Kessler, B.M. & Casals-pascual, C. (2013). PfHPRT: A New Biomarker Candidate of Acute *Plasmodium falciparum* Infection. *Journal of Medical Microbiology* **62**: 1491-1505.

Gething, P.W., Elyazar, I.R.F., Moyes, C.L., Smith, D.L., Battle, K.E., Guerra, C.A. & Hay, S.I. (2012). A Long Neglected World Malaria Map: *Plasmodium vivax* Endemicity in 2010. *PLoS Neglected Tropical Diseases* **6**: e1814.

Gething, P.W., Patil, A.P., Smith, D.L., Guerra, C.A., Elyazar, I.R., Johnston, G.L. & Hay, S.I. (2011). A new world malaria map: Plasmodium falciparum endemicity in 2010. *Malaria Journal* **10**: 378.

Grandgenett, P.M., Otsu, K., Wilson, H.R., Wilson, M.E. & Donelson, J.E. (2007). A function for a specific zinc metalloprotease of African trypanosomes. *PLoS Pathogens* **3**: 1432-1445.

Gretes, M.C., Poole, L.B. & Karplus, P.A. (2012). Peroxiredoxins in Parasites. *Antioxidants and Redox Signaling* **17**: 608-633.

Hakimi, H., Goto, Y., Suganuma, K., Ma, J., Angeles, M., Kawai, S. & Kawazu, S. (2015). Experimental Parasitology Development of monoclonal antibodies against *Plasmodium falciparum* thioredoxin peroxidase 1 and its possible application for malaria diagnosis. *Experimental Parasitology* **154**: 62-66.

Harani, M.S., Beg, M.A., Khaleeq, L., Adil, S.N., Kakepoto, G.N. & Khurshid, M. (2006). Role of ICT malaria immunochromatographic test for rapid diagnosis of malaria. *Journal of the Pakistan Medical Association* **56**: 167-171.

Hasker, E., Lutumba, P., Mumba, D., Lejon, V., Büscher, P., Kande, V. & Boelaert, M. (2010). Diagnostic accuracy and feasibility of serological tests on filter paper samples for outbreak detection of *T.b. gambiense* human African trypanosomiasis. *American Journal of Tropical Medicine and Hygiene* **83**: 374-379.

Ifeorah, I.K., Brown, B.J. & Sodeinde, O.O. (2017). A comparison of rapid diagnostic testing (by plasmodium lactate dehydrogenase), and quantitative buffy coat technique in malaria diagnosis in children. *African Journal of Infectious Diseases* **11**: 31-38.

Jain, P., Chakma, B., Patra, S. & Goswami, P. (2014). Potential biomarkers and their applications for rapid and reliable detection of malaria. *BioMed Research International* **2014**: 20.

Jimenez, A., Rees-Channer, R.R., Perera, R., Gamboa, D., Chiodini, P.L., González, I.J. & Ding, X.C. (2017). Analytical sensitivity of current best-in-class malaria rapid diagnostic tests. *Malaria Journal* **16**: 128.

Kabiri, M., Franco, J.R., Simarro, P.P., Ruiz, J.A., Sarsa, M. & Steverding, D. (1999). Detection of *Trypanosoma brucei gambiense* in sleeping sickness suspects by PCR amplification of expression-site-associated genes 6 and 7. *Tropical Medicine and International Health* **4**: 658-661.

Kanmogne, G.D., Asonganyi, T. & Gibson, W.C. (1996). Detection of *Trypanosoma brucei gambiense*, in serologically positive but aparasitaemic sleeping-sickness suspects in Cameroon, by PCR. *Annals of Tropical Medicine and Parasitology* **90**: 475-83.

Kattenberg, J.H., Versteeg, I., Migchelsen, S.J., Iveth, J., Perkins, M.D., Mens, P.F. & Schallig, H.D.F.H. (2017). New developments in malaria diagnostics Monoclonal antibodies against *Plasmodium* dihydrofolate. *mAbs* **4**: 120-126.

Keough, D.T., Hocková, D., Kreˇ, M., Cesnek, M., Hol, A., Naesens, L. & Guddat, L.W. (2010). Molecular and Biochemical Parasitology *Plasmodium vivax* hypoxanthine-guanine phosphoribosyltransferase/: A target for anti-malarial chemotherapy. *Molecular and Biochemical Parasitology* **173**: 165-169.

Kim, H., Certa, U., Dobeli, H., Jakob, P. & Hol, W.G. (1998). Crystal structure of fructose-1,6-bisphosphate aldolase from the human malaria parasite *Plasmodium falciparum*. *Biochemistry* **37**: 4388-4396.

Kim, S.H., Nam, M.-H., Roh, K.H., Park, H.C., Nam, D.H., Park, G.H. & Lim, C.S. (2008). Evaluation of a rapid diagnostic test specific for *Plasmodium vivax*. *Tropical Medicine and International Health/: TM and IH* **13**: 1495-500.

Knapp, B., Hundt, E. & Küpper, H.A. (1990). *Plasmodium falciparum* aldolase: gene structure and localization. *Molecular and Biochemical Parasitology* **40**: 1-12.

Krause, R.G.E., Hurdayal, R., Choveaux, D., Przyborski, J.M., Coetzer, T.H.T. & Goldring, J.P.D. (2017). *Plasmodium* glyceraldehyde-3-phosphate dehydrogenase: A potential malaria diagnostic target. *Experimental Parasitology* **179**: 7-19.

Krauth-Siegel, R.L., Muller, J.G., Lottspeich, F. & Schirmer, R.H. (1996). Glutathione reductase and glutamate dehydrogenase of *Plasmodium falciparum*, the causative agent of tropical malaria. *Eur J Biochem* **235**: 345-350.

Kyabayinze, D.J., Asiimwe, C., Nakanjako, D., Nabakooza, J., Counihan, H. & Tibenderana, J.K. (2010). Use of RDTs to improve malaria diagnosis and fever case management at primary health care facilities in Uganda. *Malaria Journal* **9**: 18.

Kyabayinze, D.J., Tibenderana, J.K., Odong, G.W., Rwakimari, J.B. & Counihan, H. (2008). Operational accuracy and comparative persistent antigenicity of HRP2 rapid diagnostic tests for *Plasmodium falciparum* malaria in a hyperendemic region of Uganda. *Malaria Journal* **7**: 221.

L'Ollivier, C. & Piarroux, R. (2013). Diagnosis of human nematode infections. *Expert Review of Anti-Infective Therapy* **11**: 1363-1376.

Lee, G.-C., Jeon, E.-S., Le, D.T., Kim, T.-S., Yoo, J.-H., Kim, H.Y. & Chong, C.-K. (2011). Development and evaluation of a rapid diagnostic test for *Plasmodium falciparum*, *P. vivax*, and mixed-species malaria antigens. *The American Journal of Tropical Medicine and Hygiene* **85**: 989-93.

Lee, N., Baker, J., Andrews, K.T., Gatton, M.L., Bell, D., Cheng, Q. & McCarthy, J. (2006). Effect of sequence variation in *Plasmodium falciparum* histidine- rich protein 2 on binding of specific monoclonal antibodies: Implications for rapid diagnostic tests for malaria.

Journal of Clinical Microbiology **44**: 2773-2778.

Lee, N., Baker, J., Bell, D., McCarthy, J. & Cheng, Q. (2006). Assessing the genetic diversity of the aldolase genes of *Plasmodium falciparum* and *Plasmodium vivax* and its potential effect on performance of aldolase-detecting rapid diagnostic tests. *Journal of Clinical Microbiology* **44**: 4547-4549.

Lee, S., Song, K.M., Jeon, W., Jo, H., Shim, Y.B. & Ban, C. (2012). A highly sensitive aptasensor towards *Plasmodium* lactate dehydrogenase for the diagnosis of malaria. *Biosensors and Bioelectronics* **35**: 291-296.

Lejon, V., Jamonneau, V., Solano, P., Atchade, P., Mumba, D., Nkoy, N. & Buscher, P. (2006). Detection of trypanosome-specific antibodies in saliva, towards non-invasive serological diagnosis of sleeping sickness. *Tropical Medicine and International Health* **11**: 620-627.

Li, Y., Ning, Y.S., Li, L., Peng, D.D., Dong, W.Q. & Li, M. (2005). Preparation of a monoclonal antibodies against *Plasmodium falciparum* glutamate dehydrogenase and establishment of colloidal gold-immunochromatographic assay. *Di Yi Jun Yi Da Xue Xue Bao* **25**: 435-438.

Löffek, S., Schilling, O. & Franzke, C.-W. (2011). Biological role of matrix metalloproteinases: a critical balance. *The European Respiratory Journal* **38**: 191-208.

Lutumba, P., Robays, J., Miaka, C., Kande, V., Mumba, D., Büscher, P. & Boelaert, M. (2006). Validity, cost and feasibility of the mAECT and CTC confirmation tests after diagnosis of African of sleeping sickness. *Tropical Medicine and International Health/: TM and IH* **11**: 470-8.

Makler, M.T. & Hinrichs, D.J. (1993). Measurement of the lactate dehydrogenase activity of *Plasmodium falciparum* as an assessment of parasitemia. *American Journal of Tropical Medicine and Hygiene* **48**: 205-210.

Mendis, K., Sina, B.J., Marchesini, P. & Carter, R. (2001). The neglected burden of *Plasmodium vivax* malaria. In *American Journal of Tropical Medicine and Hygiene* **64**: 97-106.

Miezan, T.W., Meda, A.H., Doua, F. & Cattand, P. (1994). Evaluation of the parasitologic techniques used in the diagnosis of human *Trypanosoma gambiense* trypanosomiasis in the Ivory Coast. *Bulletin de La Societe de Pathologie Exotique* **87**: 101-104.

Mirdha, B.R., Samantaray, J.C. & Mishra, B. (1997). Laboratory diagnosis of malaria. *Journal of Clinical Pathology* **50**: 356.

Mouatcho, J.C. & Goldring, J.P.D. (2017). Malaria rapid diagnostic tests/: challenges and prospects. *Journal of Medical Microbiology* **62**: 1491-1505.

Nagase, H., Visse, R. & Murphy, G. (2006). Structure and function of matrix metalloproteinases and TIMPs. *Cardiovascular Research* **69**: 562-573.

Nantulya, V.M. (1997). TrypTect CIATT® – a card indirect agglutination trypanosomiasis test for diagnosis of *Trypanosoma brucei gambiense* and *T. b. rhodesiense* infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**: 551-553.

Nantulya, V.M., Doua, F. & Molisho, S. (1992). Diagnosis of *Trypanosoma brucei gambiense* sleeping sickness using an antigen detection enzyme-linked immunosorbent assay. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**: 42-45.

Ngoyi, M., Matovu, E., Enyaru, J.C.K., Turck, N., Ndung, J.M., Lejon, V. & Mu, M. (2011). Matrix metalloproteinase-9 and intercellular adhesion molecule 1 are powerful staging markers for human African trypanosomiasis **16**: 119-126.

Ochola, L.B., Vounatsou, P., Smith, T., Mabaso, M.L.H. & Newton, C.R.J.C. (2006). The reliability of diagnostic techniques in the diagnosis and management of malaria in the absence of a gold standard. *The Lancet Infectious Diseases* **6**: 582-588.

Oduola, A.M., Omitowoju, G.O., Sowunmi, A., Makler, M.T., Falade, C.O., Kyle, D.E. & Milhous, W.K. (1997). *Plasmodium falciparum*: evaluation of lactate dehydrogenase in monitoring therapeutic responses to standard antimalarial drugs in Nigeria. *Exp Parasitol* **87**: 283-289.

Park, T.S., Kim, J.H., Kang, C.I., Lee, B.H., Jeon, B.R., Lee, S.M. & Kim, H.H. (2006). Diagnostic Usefulness of SD Malaria Antigen and Antibody Kits for Differential Diagnosis of vivax Malaria in Patients with Fever of Unknown Origin. *The Korean Journal of Laboratory Medicine* **26**: 241-245.

Payne, D. (1988). Use and limitations of light microscopy for diagnosing malaria at the primary health care level. *Bulletin of the World Health Organization* **66**: 621-626.

Penchenier, L., Simo, G., Grebaut, P., Nkinin, S., Laveissiere, C. & Herder, S. (2000). Diagnosis of human trypanosomiasis, due to *Trypanosoma brucei gambiense* in central Africa, by the polymerase chain reaction. *Trans R Soc Trop Med Hyg* **94**: 392-394.

Piper, R.C., Buchanan, I., Choi, Y. & Makler, M.T. (2011). Opportunities for improving pLDH-based malaria diagnostic tests. *Malaria Journal* **10**: 213.

Piper, R., LeBras, J., Wentworth, L., Hunt-Cooke, A., Houzé, S., Chiodini, P. & Makler, M. (1999). Immunocapture diagnostic assays for malaria using *Plasmodium* lactate dehydrogenase (pLDH). *American Journal of Tropical Medicine and Hygiene* **60**: 109-118.

Radwanska, M., Claes, F., Magez, S., Magnus, E., Perez-Morga, D., Pays, E. & Buscher, P. (2002). Novel primer sequences for polymerase chain reaction-based detection of *Trypanosoma brucei gambiense*. *American Journal of Tropical Medicine and Hygiene* **67**: 289-295.

Ratsimbasoa, A., Fanazava, L., Radrianjafy, R., Ramilijaona, J., Rafanomezantsoa, H. & Menard, D. (2008). Short Report: Evaluation of Two New Immunochromatographic Assays for Diagnosis of Malaria. *American Journal of Tropical Medicine and Hygiene* **79**: 670-672.

Roberto, F., Gutierrez, S., Lalu, M.M., Mariano, F.S., Milanezi, C.M., Cena, J. & Silva, J.S. (2017). Increased Activities of Cardiac Matrix Metalloproteinases Matrix Metalloproteinase (MMP)– 2 and MMP-9 Are Associated with Mortality during the Acute Phase of Experimental *Trypanosoma cruzi* Infection. *The Journal of Infectious Disease* **197**: 1468-1476

Rodríguez-Acosta, A., De Dominguez, N., Aguilar, I. & Girón, M.E. (1999). Detection of glutamate dehydrogenase enzyme activity in *Plasmodium falciparum* infection. *Indian Journal of Medical Research* **109**: 152.

Rodríguez-Acosta, A., Domínguez, N.G., Aguilar, I. & Girón, M.E. (1998). Characterization of *Plasmodium falciparum* glutamate dehydrogenase-soluble antigen. *Brazilian Journal of Medical and Biological Research* **31**: 1149-1155.

Salmani, M.P., Preeti, B.M. & Peerapur, B.V. (2011). Comparative study of peripheral blood smear and quantitative buffy coat in malaria diagnosis. *The Journal of Communicable Diseases* **43**: 57-9.

Satchell, J.F., Malby, R.L., Luo, C.S., Adisa, A., Alpyurek, A.E., Klonis, N. & Colman, P.M. (2005). Structure of glyceraldehyde-3-phosphate dehydrogenase from *Plasmodium falciparum*. *Acta Crystallographica Section D: Biological Crystallography* **61**: 1213-1221.

Shoemark, D.K., Cliff, M.J., Sessions, R.B. & Clarke, A.R. (2007). Enzymatic properties of the lactate dehydrogenase enzyme from *Plasmodium falciparum*. *FEBS Journal* **274**: 2738-2748.

Srivastava, I.K., Schmidt, M., Certa, U., Dobeli, H. & Perrin, L.H. (1990). Specificity and inhibitory activity of antibodies to *Plasmodium falciparum* aldolase. *J Immunol* **144**: 1497-1503.

Sue, G.R., Ho, Z.C. & Kim, K. (2005). Peroxiredoxins: A historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radical Biology and Medicine* **38**: 1543-1552.

Tallant, C., Marrero, A. & Gomis-Rüth, F.X. (2010). Matrix metalloproteinases: Fold and function of their catalytic domains. *Biochimica et Biophysica Acta – Molecular Cell Research* **1803**: 20-28.

Tangpukdee, N., Duangdee, C., Wilairatana, P. & Krudsood, S. (2009). Malaria diagnosis: a brief review. *The Korean Journal of Parasitology* **47**: 93-102.

Tritten, L., Matile, H., Brun, R. & Wittlin, S. (2009). A new double-antibody sandwich ELISA targeting *Plasmodium falciparum* aldolase to evaluate anti-malarial drug sensitivity. *Malaria Journal* **8**: 226.

Ugah, U.I., Alo, M.N., Owolabi, J.O., Okata-Nwali, O.D., Ekejindu, I.M., Ibeh, N. & Elom, M.O. (2017). Evaluation of the utility value of three diagnostic methods in the detection of malaria parasites in endemic area. *Malaria Journal* **16**: 189.

Wagner, J.T., Lüdemann, H., Färber, P.M., Lottspeich, F. & Krauth-Siegel, R.L. (1998). Glutamate dehydrogenase, the marker protein of *Plasmodium falciparum* – cloning, expression and characterization of the malarial enzyme. *European Journal of Biochemistry / FEBS* **258**: 813-819.

Werner, C., Stubbs, M.T., Krauth-Siegel, R.L. & Klebe, G. (2005). The crystal structure of *Plasmodium falciparum* glutamate dehydrogenase, a putative target for novel antimalarial drugs. *Journal of Molecular Biology* **349**: 597-607.

WHO. (2017). Retrieved December 21, 2017, from http://www.who.int/en/

Wilson, M.L. (2013). Laboratory diagnosis of malaria: conventional and rapid diagnostic methods. *Archives of Pathology and Laboratory Medicine* **137**: 805-11.

Wongsrichanalai, C., Barcus, M.J., Muth, S., Sutamihardja, A. & Wernsdorfer, W.H. (2007). A review of malaria diagnostic tools: Microscopy and rapid diagnostic test (RDT). *American Journal of Tropical Medicine and Hygiene* **77**: 119-127.

Wongsrichanalai, C., Namsiripongpun, V., Pornsilapatip, J., Kyle, D. & Wilde, H. (1992). Sensitivity of QBC malaria test. *The Lancet* **340**: 792-793.