Sudan University of Science and Technology
College of Graduate Studies

Evaluation of Three Different Commercial Brands of Malarial Rapid Diagnostic Tests in Khartoum State - Sudan

تقييم ثلاث علامات تجارية مختلفة لفحص الملاريا السريع في ولاية الخرطوم - السودان

A dissertation submitted in partial fulfillment of the requirements of M.Sc. degree in Medical Laboratory Science (Parasitology and Medical Entomology)

Submitted by

Zainab Ibrahim Altahir Abd Alrahman

Supervisor

Dr. Ahmed Bakheet Abd Alla

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الآية

بسم الله الرحمن الرحيم

أَفَحَسِبْتُمْ أَنَّمَا خَلَقْنَاكُمْ عَبَثًا وَأَنَّكُمْ إِلَيْنَا لَا تُرْجَعُونَ ﴿115﴾ فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۖ لَا إِلَٰهَ إِلَّا هُوَ رَبُّ الْعَرْشِ الْكَرِيمِ ﴿116﴾ وَمَنْ يَدْعُ مَعَ اللَّهِ إِلَٰهًا آخَرَ لَا بُرْهَانَ لَهُ بِهِ فَإِنَّمَا حِسَابُهُ عِنْدَ رَبِّهِ ۚ إِنَّهُ لَا يُفْلِحُ الْكَافِرُونَ ﴿117﴾ وَقُلْ رَبِّ اغْفِرْ وَارْحَمْ وَأَنْتَ خَيْرُ الرَّاحِمِينَ ﴿118﴾

صدق الله العظيم

سورة المؤمنون الآيات 115-118

Dedication

To my beloved father always and forever.

Acknowledgment

For all those who stood by my side

For my sisters, friends and my mom`s soul

For my supervisor my guide and mentor

Abstract

Malaria remains one of the most important endemic disease that threatens the mankind. The existing, accepted gold standard for diagnosing malaria is the microscopic examination of thick and thin blood smears. This method has the advantage of high sensitivity, quantifiable results, and accurate speciation, but is fairly time consuming and requires well trained staff in order to detect low parasitemia and to properly differentiate the species. The rapid diagnostic tests strips do not require the same level of training, and are also significantly faster.

This study aimed to evaluate the efficiency of Malaria rapid diagnostic tests (RDTs) by testing blood samples on three different brands and comparing the results. It was conducted in Sudan Khartoum state Bahri locality in El-ban Jadeed hospital in a period from April 2020 to June 2021. 498 blood samples were collected, 174 samples were Malaria positive which gave 34.9% as prevalence rate. 50 samples out of 174 selected randomly and each one tested on 3 different RDTs brands (Right sign, Core and Eco) results was as the follow: 27 (54%), 26 (52%) and 22 (44%) for Core, Right sign and Eco respectively.

According to parasitemia in the blood film, 15 samples found to be + all the three brands gave negative results, 12 samples + all brands gave negative results also except Right sign only 2 (16%) samples gave positive results, +++ and ++++ all brands gave positive results.

The average sensitivity was 50% while the average specificity was 49.3%, based on this study and, companies of rapid diagnostic tests should be submit to farther scrutiny by the Standards and Metrology Authority, with consideration of proper storage to give more accurate results.

ملخص الدراسة

لا تزال الملاريا واحدة من أهم الأمراض المتوطنة التي تهدد البشرية. المعيار الذهبي الحالي والمقبول لتشخيص الملاريا هو الفحص المجهري لمسحات الدم السميكة والرقيقة. تتميز هذه الطريقة بالحساسية العالية والنتائج القابلة للقياس الكمي والانتواع الدقيق، ولكنها تستغرق وقتا طويلا إلى حد ما وتتطلب موظفين مدربين تدريبا جيدا من أجل الكشف عن انخفاض الطفيليات والتمييز بين الأنواع بشكل صحيح. لا تتطلب شرائط الاختبارات التشخيصية السريعة نفس المستوى من التدريب، كما أنها أسرع بكثير.

هدفت هذه الدراسة للتحقق من مدى فعالية فحص الملاريا السريع وذلك عن طريق إختبار عينات دم إيجابية الملاريا بعد حساب عدد الطفيليات على ثلاثة شركات منتجة لشرائط الفحص السريع وتمت مقارنة النتائج.

أُجريت هذه الدراسة في الخرطوم محلية بحري مستشفى البان جديد وذلك في الفترة من ابريل ٢٠٢١ الى يونيو٢٠٢٢ تم جمع ٤٩٨ عينة دم كان منها ١٧٤ عينة إيجابية الملاريا وهذا شكل معدل إصابة بنسبة ٣٤,٩٪ . من أصل ١٧٤ عينة إيجابية الملاريا تم إختيار ٥٠ عينة عشوائياً أُختُبرت كل واحدة منهم على ثلاثة شركات مختلفة (كور , رايت ساين و إيكو) وكانت النتائج الايجابية كالتالي : ٢٧ (٥٤٪) ٢٦ (٥٢٪) و ٢٢ (٤٤٪) من أصل ٥٠ عينة لكل من كور رايت ساين وإيكو على التوالي.

بناءاً على عدد الطفيليات في مسحات الدم وُجِدت ١٥ عينة + أعطت الثلاثة شركات نتائج سلبية وعندنا كانت ++ ولم تعطي جميع الشركات نتائج عدا رايت ساين بمعدل ١٦٪ عينتان من اصل ١٢ عينة ، +++ كانت ١٨ مسحة دم ، النتائج الإيجابية علي الفحص السريع كانت جميعها إيجابية تقريباً على الشركات الثلاثة كما انطبقت نفس النتيجة عندما كانت قراءة شرائح مسحات الدم ++++ .

متوسط حساسية الشركات كانت ٥٠٪ بينما متوسط الخصوصية ٤٩,٣٪. وفقاً لهذه الدراسة وبعد الرجوع لتوصيات منظمة الصحة العالمية فإن شركات شرائح الفحص السريع للملاريا يجب ان تخضع لمزيد من التدقيق من قبل هيئة المواصفات والمقاييس مع مراعاة صحة التخزين حتى تعطي نتائج أكثر دقة وفعالي.

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Chapter One

1.1 Introduction

Malaria is caused by *Plasmodium* parasites. The parasites are spread to people through the bites of infected female *Anopheles* mosquitoes, called "malaria vectors". There are 4 *Plasmodium* species that cause malaria in humans (*Plasmodium falciparum*, *P. vivax*, *P. ovale and P. malarae)*, two of these species – *P. falciparum* and *P. vivax* – pose the greatest threat (World Health Organization, 2014).

Malaria is an acute febrile illness. In a non-immune individual; symptoms usually appear 10–15 days after the infective mosquito bite. The symptoms - fever, headache, and chills may be mild and difficult to recognize as malaria. If not treated within 24 hours, *P. falciparum* malaria can progress to severe illness, often leading to death (WHO, 2020).

Children with severe malaria frequently develop one or more of the following symptoms: severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria. In adults, multi-organ failure is also frequent. In malaria endemic areas, people may develop partial immunity, allowing asymptomatic infections to occur (WHO, 2020).

In 2018, *P. falciparum* accounted for 99.7% of estimated malaria cases in the WHO African Region 50% of cases in the WHO South-East Asia region, 71% of cases in the Eastern Mediterranean and 65% in the Western Pacific. *P. vivax* is the predominant parasite in the WHO region of the Americas, representing 75% of malaria cases. In 2018, there were an estimated 228 million cases of malaria worldwide. The estimated number of malaria deaths stood at 405 000 in 2018. Children aged under 5 years

are the most vulnerable group affected by malaria; in 2018, they accounted for 67% (272 000) of all malaria deaths worldwide (WHO, 2020).

The WHO African region carries a disproportionately high share of the global malaria burden. In 2018, the region was home to 93% of malaria cases and 94% of malaria deaths. Total funding for malaria control and elimination reached an estimated US$ 2.7 billion in 2018. Contributions from governments of endemic countries amounted to US$ 900 million, representing 30% of total funding (WHO, 2020).

In Sudan in 2015, 586,827 confirmed cases were reported from public health facilities out of the estimated 1,400,000 cases (970,000; 1,900,000). As well, 868 deaths were reported out of the estimated 3,500 deaths (130; 6800). The reported malaria cases represent 8.7% and 12.2% of the total outpatient attendance and of hospital admissions respectively. The disease proportional mortality was 4.3% in 2015 putting malaria as one of the main causes of death in Sudan. Results of the Sudan Malaria Indicators Survey in 2016, showed an overall parasite prevalence of 5.9%. The prevalence is range between 20% in Central Darfur State. In South Darfur, West Darfur, Blue Nile and South Kordofan states the prevalence approached or exceeded 10%. The prevalence correlates with age, as children are 3 times more likely to get malaria than adults. Apparently, there was no difference between male and female . Similarly, the lowest economic class is at higher risk. Internally displaced people and refugee camps reported prevalence doubled that in rural areas and 3 times higher than that in urban areas. The main species is the *P. falciparum* representing 87.6% of cases. However, the *P. vivax* unexpectedly reaches 8.1% and mixed infection (*P. falciparum* & *P. vivax*) approached 5%. *P. vivax* alone plus mixed infection exceeded 15% in North Darfur, West Darfur, South Darfur, River Nile and Khartoum

states. The main vector species is *Anopheles arabiensis* besides *An. Gambia* and *An. funestus* (Oladapo,2009).

1.2 Rationale

Malaria can be fatal if it is not diagnosed properly as soon as possible. It can cause a serious damage to the human body if it did not kill him. Sudan is an open market to all over the world and since it is one of the 3rd world countries the standards are sometimes inaccurate as it should be according to the standards and metrology authority which give tens of company the opportunity to manipulates the material which at the end give inaccurate or false results. Once the diagnosis is the key to treat and defeat malaria, we have to make sure that all equipment's work properly. So, this aim to give a picture about the accuracy of the RDT compared to the blood film in order to help evaluation the market.

1.3 Objectives

1.3.1 General objective

Evaluation of Three Different Brands of Malarial Rapid Diagnostic Tests in Khartoum State Sudan.

1.3.2 Specific objectives

-To estimate the prevalence of Malaria in study area

-To determinate the parasitemia among participants blood sample

-To evaluate RDT sensitivity and specificity

-To compare between the parasitemia and RDT sensitivity

Chapter two

2. Literature review

2.1 History of malaria

Malaria is caused by infection with protozoan parasites belonging to the genus *Plasmodium* transmitted by female *Anopheles* species mosquitoes. Our understanding of the malaria parasites begins in 1880 with the discovery of the parasites in the blood of malaria patients by Alphonse Laveran. The sexual stages in the blood were discovered by William MacCallum in birds infected with a related haematozoan, *Haemoproteus columbae*, in 1897 and the whole of the transmission cycle in Culicine mosquitoes and birds infected with *Plasmodium relictum* was elucidated by Ronald Ross in 1897. In 1898 the Italian malariologists, Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli, Camillo Golgi and Ettore Marchiafava demonstrated conclusively that human malaria was also transmitted by mosquitoes, in this case Anophelines. The discovery that malaria parasites developed in the liver before entering the blood stream was made by Henry Shortt and Cyril Garnham in 1948 and the final stage in the life cycle, the presence of dormant stages in the liver, was conclusively demonstrated in 1982 by Wojciech Krotoski (Cox, 2010). In 1976, Trager and Jensen cultured malaria parasites in-vitro for the first time (Arora *et al*, 2010).

2.2 Biology of malaria parasite

In humans, malaria parasites grow and multiply first in the liver cells and then exponentially in the red blood cells. It is the blood stage of the parasite lifecycle that causes the symptoms of malaria in humen.

Malaria is usually classified as asymptomatic, uncomplicated or severe.

Asymptomatic malaria can be caused by all *Plasmodium* species; the patient has circulating parasites but no symptoms.

Uncomplicated malaria can be caused by all *Plasmodium* species. Symptoms generally occur 7-10 days after the initial mosquito bite. Symptoms are non-specific and can include fever, moderate to severe shaking chills, profuse sweating, headache, nausea, vomiting, diarrhoea and anaemia, with no clinical or laboratory findings of severe organ dysfunction.

Severe malaria is usually caused by infection with *Plasmodium falciparum*, though less frequently can also be caused by *Plasmodium vivax* or *Plasmodium knowlesi*. Complications include severe anaemia and endorgan damage, including coma (cerebral malaria), pulmonary complications and hypoglycaemia or acute kidney injury. Severe malaria is often associated with hyperparasitaemia and is associated with increased mortality (Warrell, 2017).

2.3 Distribution of malaria:

2.3.1 Distribution of malaria in the world:

Malaria is one of the most significant infectious diseases of humans. According to the WHO (2000, 2004), the disease is currently endemic in more than 100 countries or territories, mainly in sub-Saharan Africa, Asia, Oceania, Central and South America, and in the Caribbean. About 2.4 billion people (40% of the world's population) live in malarious regions. Fig. 2:1 shows the Distribution of malaria in the world (CDC, 2015). The annual incidence of malaria worldwide is estimated to be 300–500 million clinical cases, with about 90% of these occurring in sub-Saharan Africa (mostly caused by *P. falciparum*). Malaria alone or in combination with other diseases kills approximately 1.1–2.7 million people each year, including 1 million children under the age of five years in tropical Africa.

About 7000 cases of imported malaria were reported in Europe in the period from 1985 to 1995, whereby the data are incomplete (**Kayser**, 2005).

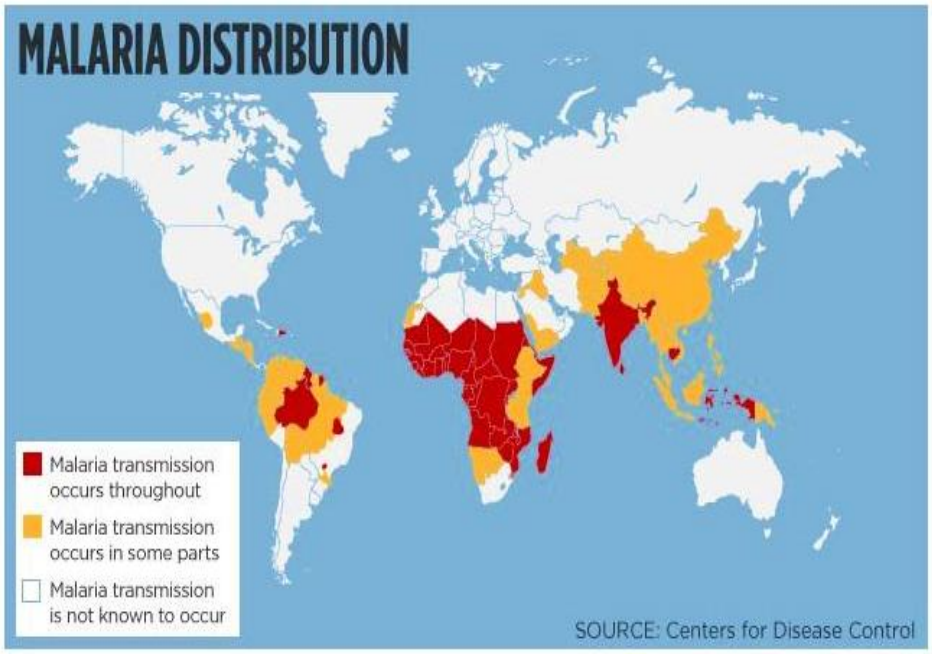

Figure (2-1): Distribution of malaria in the world (CDC, 2015).

2.3.2 Malaria distribution in Sudan:

Malaria constitutes a major public health problem in Sudan. Almost, 75% of population is at risk of developing malaria. In 2015, 586,827 confirmed cases were reported from public health facilities out of the estimated 1,400,000 cases (970,000; 1,900,000). As well, 868 deaths were reported out of the estimated 3,500 deaths (130; 6800). The reported malaria cases represent 8.7% and 12.2% of the total outpatient attendance and of hospital admissions respectively. The disease proportional mortality was 4.3% in 2015 putting malaria as one of the main causes of death in Sudan. Results of the Sudan Malaria Indicators Survey in 2016 (Sudan MIS, 2016), showed an overall parasite prevalence of 5.9%. The prevalence

is range between <1 in Red Sea, Northern, River Nile and Khartoum States to >20% in Central Darfur State **(Gibreel**, 2015).

2.4 Epidemiology of *Plasmodium* infection:

Malaria exists where effective Anopheline vectors breed in nature and where human carries of the sexual forms of the parasites are available to these mosquitoes. In a few parts of the world Anopheline have been present in the absence of malaria, but these areas are limited. As a result of control and eradication procedures many former malarious are now clear because the Anopheline population has been reduced or eliminated. In those areas where vectors are still great malaria persists, the epidemiology of the disease is the resultant of various factors. (Fakhreldin *et al.*, 2003).

2.5 *Plasmdium*s life cycle:

The life cycle of malaria parasites is extremely complex and requires specialized protein expression for survival in both the invertebrate and vertebrate hosts. These proteins are required for both intracellular and extracellular survival, for the invasion of a variety of cell types and for the evasion of host immune responses. Once injected into the human host, *P. falciparum* and *P. malariae* sporozoites trigger immediate schizogony, whereas *ovale P.* and *vivax P.* sporozoites may either trigger immediate schizogony or lead to delayed schizogony as they pass through the hypnozoite. The life cycle of the malaria parasite is shown in (Fig. 2.2) and can be divided into several stages, starting with sporozoite entry into the bloodstream (Tuteja, 2007).

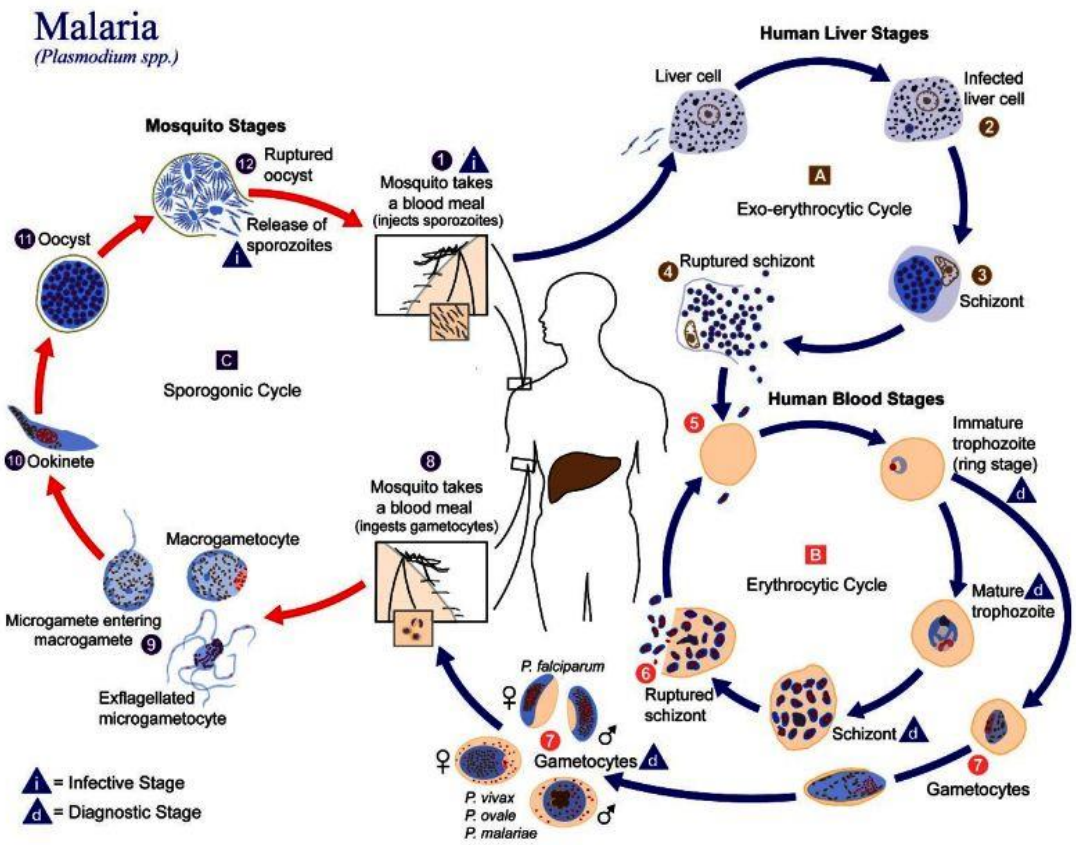

Figure (2.2): Malaria parasite life cycle.

The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host❶. Sporozoites infect liver cells ❷and mature into schizonts ❸, which rupture and release merozoites❹. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later). After this initial replication in the liver (exo-erythrocyticschizogony🅐), the parasites undergo asexual multiplication in the erythrocytes (erythrocyticschizogony🅑)Merozoites infect red blood cells ❺. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites❻. Some parasites differentiate into sexual erythrocytic stages (gametocytes)❼. Blood stage parasites are responsible for the clinical manifestations of the disease (CDC, 2012).

The life cycle in humans begins with the introduction of sporozoites into the blood from the saliva of the biting mosquito. The sporozoites are taken up by hepatocytes within 30 minutes (Levinson, 2014). Parasites develop in the liver over the next 7-10 days (pre-erythrocytic stage), with nuclear division to form schizonts. Hepatic infection is asymptomatic and may last from about 6 days to several weeks. When hepatocytes rupture, schizonts release into the bloodstream thousands of merozoites that in turn invade erythrocytes. In *P. vivax* and *P. ovale* malaria, some parasites may become dormant in the liver (hypnozoites) and emerge at a later stage (up to 2 years or more after leaving an endemic area) to cause a relapse of disease. Delayed prepatent *P. vivax* infections also occur, with incubation periods of up to 6-9 months in some returned travellers (Biggs and Brown, 2001). The next stage of development, called the erythrocytic or blood stage, is initiated when exo-erythrocytic merozoites from the liver invade red blood cells (RBCs). Merozoites of *P. falciparum* can infect RBCs of all ages, whereas those of *P. vivax* and *P. ovale* infect reticulocytes and those of *P. malariae* invade only older RBCs. Shortly after merozoites are released from hepatocytes, they invade RBCs and over a period of 2 or 3 days, develop asexually. The stages of asexual development include the ring (early trophozoite), trophozoite and schizont stages. At maturation, the schizont bursts and releases merozoites into the blood circulation. Most of the released merozoites re-invade a new erythrocyte, thereby repeating their asexual life cycle (Eichinger, 2009).

After a series of erythrocytic cycles, some merozoites after entering into RBCs, instead of developing into trophozoites, they transform into sexual forms called as gametocytes (Sastry and Sandhya Bhat K MD, 2014).

The sexual phase (sporogony) results when a mosquito draws infected RBCs into her stomach. In the stomach, the microgametocyte releases spermlike gametes that fertilize the larger macrogametes. The resultant diploid cell (oocyst) implants into the stomach wall of the mosquito and undergoes multiple meiotic divisions, releasing sporozoites that migrate to the salivary glands and lodge there. This event completes the sexual cycle and makes the sporozoites available for infecting the next victim (Talaro and Talaro, 2002).

2.6 Laboratories diagnosis:

At the present time there are a limited number of methods for the diagnosis of malaria. Conventional methods include clinical diagnosis by history and physical examination, empirical/syndromic diagnosis (mainly the presence of fever in endemic areas), and use of light microscopy to examine stained peripheral blood smears (Wilson, 2013**)**.

2.6.1 Empiric/Syndromic diagnosis

One widely used method to diagnose malaria is empiric/syndromic diagnosis, in which the diagnosis is made on the basis of clinical history, signs, and/or symptoms. In many endemic areas without adequate diagnostic capacity, patients with a febrile illness are likely to receive the diagnosis of malaria. There are a number of pitfalls associated with this approach. First, there is significant clinical overlap among febrile illnesses; fever alone is too nonspecific to make any particular diagnosis. Second, co infections can and do occur, and treatment for one obviously does not treat the other. Third, malaria parasitemia can occur that is not the cause of the febrile illness. Last, relying on clinical diagnosis alone results in treatment of patients with antimalarial drugs for illnesses other than malaria. The

WHO recommends against this practice when and where malaria diagnostic tests are available (WHO, 2011).

2.6.2 Microscopic slide examination

In most endemic areas, microscopic slide examination of peripheral blood remains the most widely used test as well as the gold standard for detecting malaria parasitemia. The WHO (2011) report indicates that in 2010 there were a total of 165 million microscopic slide examinations worldwide. Estimates of diagnostic sensitivity of microscopic slide evaluation vary according to the type of infecting species, geographic area, and other factors, but in general diagnostic sensitivity is thought to be no higher than 75%. This figure is based on the rate of detection of parasitemia in patients with clinical malaria. For patients with non-*falciparum* malaria, low-level parasitemia, or partial immunity, or those who have been partially treated for malaria, the diagnostic sensitivity is likely to be even lower than 75%. Even so, microscopy offers significant advantages over other methods, and, where it can be done correctly and with good quality assurance, it remains the gold standard against which other methods are compared.

Microscopy is based on examination of both thick and thin films made from the same sample of peripheral blood. Thin films are prepared in the same way as for any peripheral blood smear. A number of different stains can be used, but it is important to remember that not all stains allow detection of some of the characteristic features of malaria (eg, Schüffner dots). It is also important to remember that stains can vary in quality and consistency, and that adequate quality control and experience both are needed to provide optimal stains. Although thick films are more sensitive for detecting the presence of malaria parasites, they are not very useful in speciating the parasites, which should be done using the thin films. Even though the technology of microscopy is simple and straightforward,

making and interpreting malaria smears requires adequate training and experience.

The diagnostic advantages of microscopy are that it permits definitive identification of infecting species as well as mixed infections; can be used to determine the magnitude of parasitemia; can be used for serial examinations to monitor the efficacy of therapy; requires little laboratory infrastructure; and is comparatively inexpensive. Microscopic slide examination does have diagnostic disadvantages, including it does not detect very low parasitemias; errors in interpretation are most common with either very low or very high parasitemias (for which accurate diagnosis is very important); mixed infections are often missed; and it is not as useful in areas without endemic malaria because of the inability of persons reading smears to remain sufficiently competent to make accurate and reproducible diagnoses.

Microscopic slide examination also has nondiagnostic advantages and disadvantages. Among the advantages are that the microscope and trained personnel can be used to diagnose other infectious diseases, slides can be retained to create a permanent record for quality control, excess microscope slides can be reused or recycled, and the method requires only minimal laboratory infrastructure. Among the nondiagnostic disadvantages are that the method is labor intensive; it is relatively slow (particularly for thick films); the variable stains and methods result in variable smear quality; the method requires adequate training; and for an area that previously did not use the method, acquiring the necessary equipment and training personnel can be expensive. The difficulty in learning to interpret malaria smears is generally overstated as a limitation, as even individuals without a laboratory background can be trained to read smears in a reasonable amount of time and with good success.

2.6.3 Rapid diagnostic tests

A congress of the WHO produced a document entitled *New Perspectives in Malaria Diagnosis* (WHO, 2000). Certain recommendations were presented on what non-microscopic rapid diagnostic tests (RDT) should provide. The document concluded that results from these test devices should be at least as accurate as results derived from microscopy performed by an average technician under routine field conditions. Other criteria included the sensitivity, which should be above 95% compared to microscopy, and the detection of parasitemia, such that levels of 100 parasites/μl (0.002% parasitemia). Should be detected reliably with a sensitivity of 100%. Quantitative or semi quantitative information on parasite densities in circulating blood was considered essential. Other essential criteria suggested were the ability to distinguish viable parasites from parasite products such as antigens or nucleic acids not associated with viable organisms and also to indicate the prediction of treatment outcomes or resistance to common antimalarial drugs.

These objectives are demanding for scientists and manufacturers alike, and the extent to which they have been or can be achieved, along with economic considerations, will provide the scientists involved in the development of these RDT for malaria with a number of exciting challenges.

Immunochromatography relies on the migration of liquid across the surface of a nitrocellulose membrane. Immunochromatographic tests are based on the capture of parasite antigen from peripheral blood using monoclonal antibodies prepared against a malaria antigen target and conjugated to either a liposome containing selenium dye or gold particles in a mobile phase. A second or third capture monoclonal antibody applied

to a strip of nitrocellulose acts as the immobile phase. The migration of the antigen-antibody complex in the mobile phase along the strip enables the labeled antigen to be captured by the monoclonal antibody of the immobile phase, thus producing a visible colored line. Incorporation of a labeled goat anti-mouse antibody capture ensures that the system is controlled for migration. Migration depends on several physical characteristics of the component reagents, primarily the porosity of the membrane controlling the flow rate and the components of the buffer solution used to transport the labeled antigen-antibody complex in the lysed blood sample (Fig.2.3).

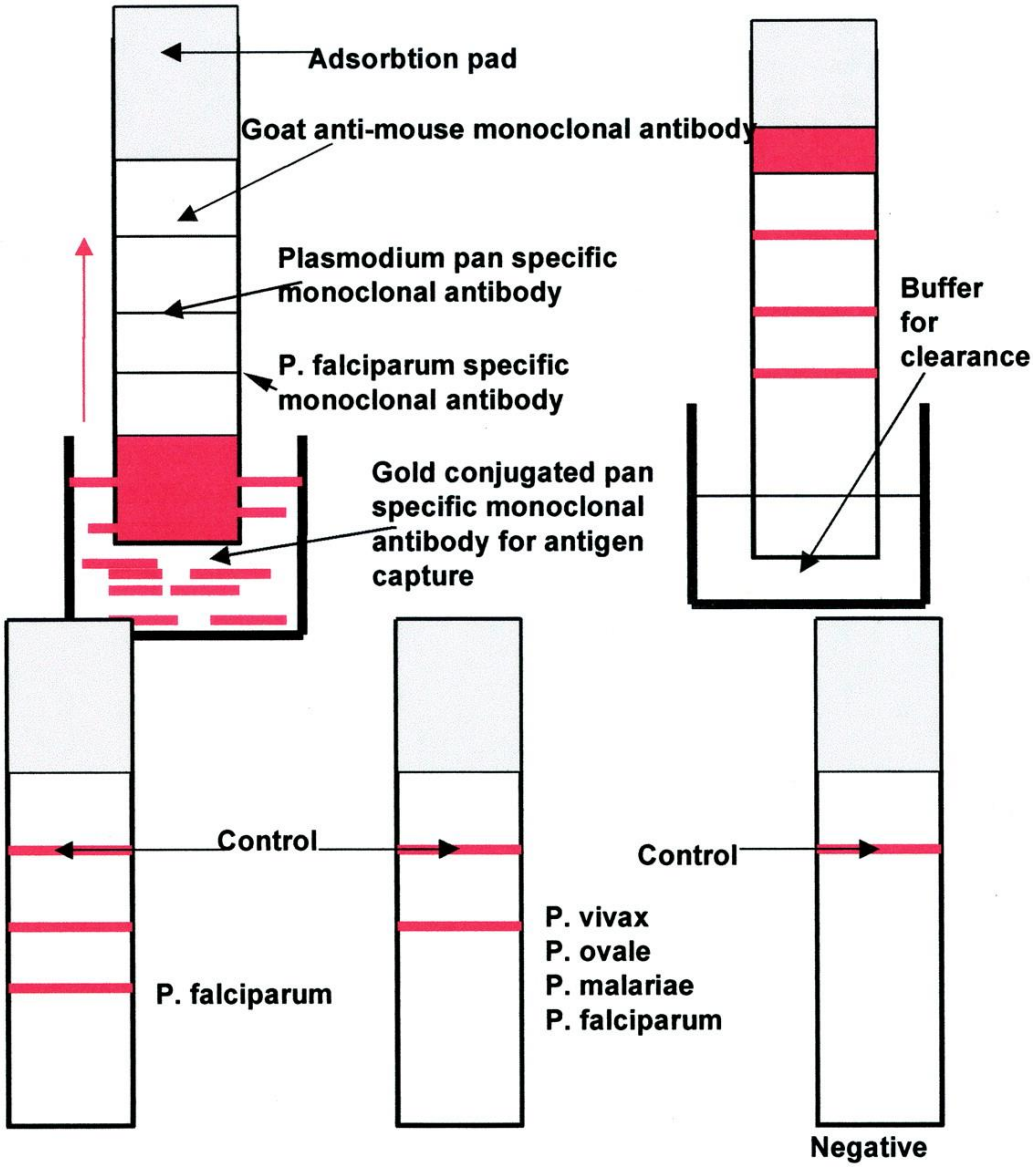

Figure (2.3): Principle of immunochromatographic RDT for malaria.

Chapter three

Materials and methods

3.1 Study design:

It is a cross sectional based on hospital study.

3.2 Study area:

This study took place in El-Ban Jadeed hospital in Bahri locality Elkhartoum state in a period from April 2020 to June 2021.

3.3 Study population:

All patients suspected of Malaria and came with Malaria test request to El-Ban Jadeed hospital, all negative blood film results were excluded.

3.4 Sampling technique:

2ml of blood sample were taken from the patient, 1 ml to make blood smear (thick and thin blood film), and 1ml to the raped diagnostic tests.

3.5 Sample size:

The sample size was 498 samples taken from El-Ban Jadeed hospital during the study period.

3.6. Population Criteria:

3.6.1 Inclusion criteria:

All patients with Malaria positive.

3.6.2 Exclusion criteria:

Patients with Malaria negative.

3.7 Methods:

3.7.1 Collection of blood samples:

A total of 2 ml of venous blood were collected and divided into 1 ml for the blood film and 1 ml for the RDT.

3.7.2 Preparation and staining of blood smears:

Three drops of collected blood were placed in clean and dry slide (about 2 cm from edge of slide) and then stirred by a corner of another clean and dry slide until appropriate thick smear obtained, the smear was left to dry. Drop of blood was placed on the middle of clean and dry slide and by edge of another slide placed just in front of the drop of blood and the spreader turned until it touched the drop of blood, then blood allowed to run along the edge of spreader, and then spreader was pushed forward to the end of the slide with suitable speed. The smear was left to dry. All thick and thin blood films were stained using Giemsa stain. Only thin films were fixed with methanol for 1-2 minutes the slides were covered with 10% Giemsa solution for 10 minutes. All slides were washed using clean water and allowed to air dry.

3.7.3 Examination of blood films:

The slides were examined using light microscope (Olympus x100 oil immersion lenses).The number of parasites were counted and reported by using the following grading according to WHO program :

- 1- 10 parasites per 100 thick film fields +.
- 11- 100 parasites per 100 thick film fields ++.
- 1- 10 parasites per thick film field +++.
- 11- 100 parasites per thick film field ++++.

3.7.4 RDTs (ICT for malaria):

Test principle:

Malaria Antigen *Plasmodium falciparum Plasmodium vivax* test cassette contains a membrane strip which is pre coated with a monoclonal antibodies two separate lines. One monoclonal antibodies (test line *P.f*) are specific to the HRP-II (Histadine rich protein II) of *Plasmodium falciparum*, and other monoclonal antibodies (test line *P.v.*)Are specific to the PLDH (*Plasmodium* lactate .dehydrogenase) of *Plasmodium vivax.*

Procedure:

Kit components and specimen were allowed to reach room temperature prior to testing. The test device was removed from foil, lace it on a flat, dry surface. For whole blood specimen a pipette was used to transfer 5µl of whole blood to the specimen well, then added 4 drops of assay diluents vertically in square assay diluents well, after that waited for a minimum of 15 minutes (up to 30) and the result read.

Interpretation of result:

- Positive: two or three distinct colored lines appear.
- *P.falciparum* or mixed malaria infection: one line appears in the control region, one line appear in *P.v*. Line region and one line appears in *P.f* Line region.
- *P.falciparum* infection: one line appears in the control region, and one line appears in *P.f*. region.
- *P.vivax* infection: one line appears in the control region, and one line appears in *P.v.* region.

Negative: only one colored line appears in the control region.

Invalid: control line fail to appear.

3.8 Ethical approval:

Approval was taken from the college of Medical Laboratory Science committee – Sudan University of Science and Technology.

3.9. Statistical analysis:

Data were analyzed using Statistical Package for the Social Sciences program (SPSS program version 20).

Chapter Four

Results

Out of 498 collected blood samples from Malaria suspected patients. 174 were found Malaria positive. The prevalence according to below equation was 34.9 % as showed in table (4.1).

$$\textbf{Prevalence \%} = \frac{\textbf{Total number of cases}}{\textbf{Total population}} \times \mathbf{100}$$

Table (4.1) prevalence of Malaria among study population:

Number of Examined Samples	Positive samples	Prevalence %
498	174	34.9%

50 samples were selected randomly from the 174 positive samples and tested on three different RDTs brands then compared according to their blood film parasitemia. The result showed that, Core had the highest positive score (54%) which was 27 samples out of the 50, when Eco had the lowest (44%) 22 samples and the only invalid sample was belonging to the same brand (Eco) as showed in table (4.2).

Table (4.2): RDT results:

RDT	Positive	Negative	Invalid
Eco	22 (44%)	27(54%)	1(2%)
Core	27(54%)	23(46%)	0(0%)
Right sign	26(52%)	24(48%)	0(0%)
Average%	**50%**	**49.3%**	**0.6%**

All RDTs brands (Eco, Core and Right sign) gave negative results when the parasitemia was +. When parasitemia was ++ Eco and Core RDTs gave a negative result also except Right sign one sample gave a positive result. When the blood film parasitemia was +++ and ++++ almost all the RDTs gave positive results.

Table (4.3): RDT positive result according to parasitemia

Parasitemia	Number of samples	Positive ECO (%)	Positive Core (%)	Positive Right sign (%)
+	10	0 (0)	0 (0)	0 (0)
++	12	0 (0)	0 (0)	2 (16)
+++	18	14 (77.7)	18 (100)	14 (77.7)
++++	10	8 (80)	8 (80)	10 (100)

The sensitivity and specificity for the three RDTs brands counted according to the equation below. It showed that Core had the highest sensitivity among the three while Eco had the best specificity as mentioned in table (4.4).

$$\boldsymbol{sensitivity} = \frac{\text{positive cases of tested techniqe (TP)}}{\text{positive cases of referance techniqe (TP + FN)}}$$

$$\boldsymbol{specificity} = \frac{\text{negative cases of tested techniqe (TN)}}{\text{negative cases of referance techniqe (TN + FP)}}$$

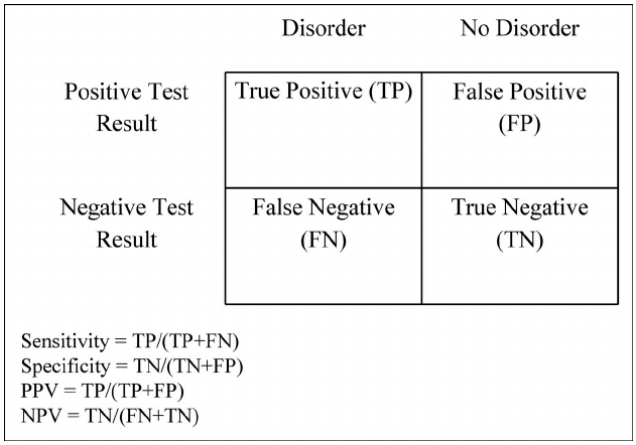

Table (4.4): sensitivity and specificity of RDTs result

RDT	Sensitivity	Specificity
Eco	44%	54%
Core	54%	46%
Right sign	52%	48%
Average	**50%**	**49.3%**

Chapter five

Discussion, Conclusion, and Recommendation

5.1 Discussion

Malaria causes a major public health problem in people living in the highly affected areas of Sudan. Evidences suggested that malaria examination techniques are altered in malaria-infected patients. This study was attempted to compare the infection rate of *Plasmodium* among Sudanese by using various techniques include blood film and RDT, the result revealed significant variation of prevalence rate and intensity of infection.

Overall prevalence rate of *Plasmodium* (34.9%) in the study group, the result was different to Han *et al*. (2017), which was a comparison of microscopy and PCR for detection of human *Plasmodium* species and *P. knowlesi* in southern Myanmar which the percentage of infection was (67.8%) and that maybe because of the high accuracy of PCR to ICT. On the other hand it was close result to Nkrumah *et al.* (2011), when there prevelance was 40.7%.

Ashton *et al.* (2010) in Ethiopia gave a quite different results when they tested the performance of three multi-species rapid diagnostic tests for diagnosis of Malaria all three RDTs were equally sensitive in detecting *P. falciparum* or mixed infection (85.6%) but in this study the sensitivity was variable between (44% and 52%) which is low when it compared to the previos study and that might be due to manufacturing reasons and quality control weakness.

The result of RDT affected by parasitemia and Gillet *et al*. (2011) study assure that when a total of (11.6%) samples showed *P. falciparum*, (10.5%) had high parasitaemia and 76 were available for prozone testing. None of the two Pf-pLDH RDTs, but all six HRP-2 RDTs showed prozone, at

frequencies between 6.7% and 38.2%. Negative and faint HRP-2 lines accounted for (3.8%) and (14.4%) of the 104 prozone results in two RDT brands. For the most affected brand, the proportions of prozone with no visible or faint HRP-2 lines were 10.9%, 1.2% and 0.1% among samples with high parasitaemia, all positive samples and all submitted samples respectively.

According to a study conducted by Elsheikh *et al*. (2019) in Sudan microscopy had higher sensitivity had RDT (88.24%) but RDTs specificity was higher than microscopy specificity (44.8%) which similar to this study.

Another study performed by Ajakaye *et al*, (2020). In Nigeria. The overall prevalence of malaria by RDT was 55.2%, while the sensitivity and specificity compared to light microscopy was 69.08% and 66.67% respectively which is slightly different from this study since sensitivity was only 50% and the specificity was 49.3%.

A study done by Nkrumah *et al.* (2011) in Ghana, this study performed to evaluate two different rapid field tests for malaria and compared to geimsa stain method. Compared to the reference standard, the sensitivities of the two brans were 100% and 97.2%, and the specificities were 97.4% and 93.6% and that consider very high when it compared to sensitivity and specificity of this study probably because of low standers.

On the contrary a study performed in 2018 prevalence of malaria in the study population was 40.32% to be positive for microscopy. Overall, the test recorded a sensitivity and specificity of 85.33% and 95.05% respectively. But the sensitivity and specificity of the RDT increased as parasite densities increased which is the same consideration of this research.

Bendezu *et al.* (2010) A total of 332 symptomatic malaria patients there were 234 negative and 98 positive slides *e.i* prevalence (29.5%),When microscopy was used as gold standard, RDT sensitivity 53.5%, Specificity 98.7%. Parascreen™ (RDT brand) had a low sensitivity (12.5%) for detection of P. vivax infections at parasite densities below 99 parasites/µl, which increased for the detection of parasite densities between 99 and 500 parasites/µl (sensitivity of 60%). For P. falciparum infections, parasite densities below 1,000 parasites/µl were detected with less sensitivity (25%) than parasite densities > 1,000 parasites/µl (sensitivity between 66.7% and 75.0%), those figures confirmed what this study had reached.

5.2 Conclusion

In conclusion, the results obtained from this study demonstrate that the prevalence at that period of time was not significant.

Malaria RDTs which were involved in this study showed performance below WHO recommendations for sensitivity compared to ‘gold standard microscopy’.

Also there was a direct relationship between the parasite count and the efficiency of the RDTs.

5.3 Recommendations

- ❖ Not to use Malaria RDTs as routine daignosis for Malaria in state of microscopy whice is concedered as golden standered with a well trained staff.
- ❖ The concerned authoritied should take good care of national standerds for RDTs company and should not allow them to invest unless the comply.
- ❖ Periodically assesment for RDTs brands in the local markets to insure the effeciency and the validity.
- ❖ Proper storage must be considered very well for both sellers and lab tichnicians to its essensial role in the results.

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Appendix