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RESEARCH ARTICLE

DIFFERENT METHODS FOR DIAGNOSING MALARIA DISEASE

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ABSTRACT

Malaria is a serious global health problem that is responsible for nearly one million deaths eachyear. In this emergency situation, prompt and effective diagnostic methods are essential for the management and control of malaria. This paper reviews different methods for diagnosing malaria disease.

INTRODUCTION

Malaria is a serious global health problem, causing widespread sufferings and deaths particularly in Africa and south Asia. In 2010, about 3.3 billion people which are half of the world populations are at risk of malaria. In addition, this disease has caused the death of an estimation of 655,000 people in 2010, with 86% of the victims are children under five years of age [1].

Malaria sometimes is caused by protozoan parasites of the genus *Plasmodium*. Malaria is caused by a peripheral blood parasite of the genus *Plasmodium*. The genus *Plasmodium* has five species that can cause human infection namely *P. falciparum* , *P.vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* [2]. The most serious and sometimes fatal type of malaria is caused by *Plasmodium falciparum*.

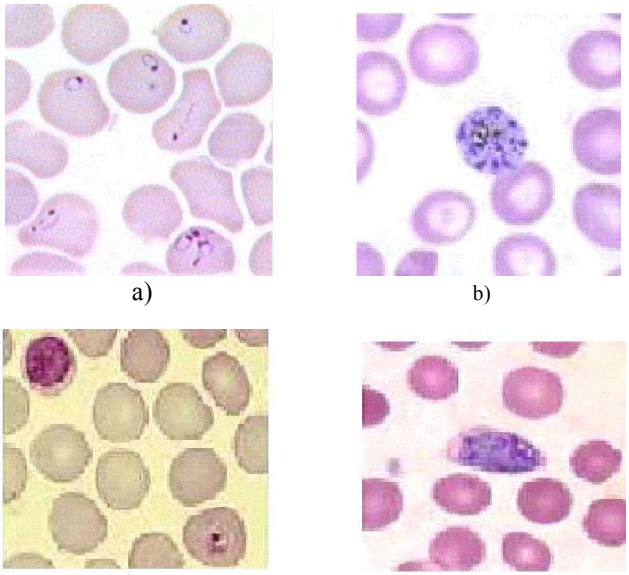
Prompt and accurate diagnoses of malaria infection are the main keys to control and cure this disease effectively. Currently, the most economic and reliable diagnosis which is based on microscopic examination of blood slide, especially based on the thin blood smear, still remains the gold standard for laboratory diagnosis of malaria [3]-[5]. In general, detection of the presence of malaria parasites in the examined blood slide is one of the most important tasks in malaria diagnosis [6]. The procedure is performed manually by expert microbiologists by searching for the parasites in blood slide using a light microscope [4], [5].

During malaria diagnosis, the presence of the parasites is recognizable by their physical features as well as the appearance of the red blood cells (RBCs) that they have infected [7].

The definitive diagnosis of malaria infection is done by searching for parasites in blood slides (films) through a microscope. In peripheral blood sample visual detection and recognition of Plasmodium is possible and efficient via a chemical process called (Giemsa) staining. The staining process slightly colorizes the red blood cells (RBCs) but highlights Plasmodium parasites, white blood cells (WBC), and platelets or artefacts. The detection of Plasmodium requires detection of the stained objects. However, to prevent false diagnosis the stained objects have to be analyzed further to determine if they are parasites or not. In the figure1 there are four types of human malaria – Plasmodium falciparum, P. vivax, P. malariae, and P. ovale. P. falciparum and P. vivax are the most common. P. falciparum is by far the most deadly type of malaria infection.

Considerable research efforts were made to develop automatic malaria detection systems on thin blood smears. For example, some scientists employed k-nearest neighbour classifier to classify parasite species. The combination of various features was used including histogram, Hu moments, relative shape measurement, and color auto-correlogram. Then few reported the use of feed forward backpropogation neural networks to detect malaria parasites on thin blood film; then classify the

parasites into four species (P. falciparum, P. vivax, P. ovale and P. malariae). Others used multilayer perceptron (MLP) network to classify the malaria parasites into three species (P. falciparum, P. vivax and P. malariae). They proposed six features (size of infected red blood cell (RBC) per size of normal RBC, shape of the parasite, numbers of the parasite's chromatin, numbers of parasite per RBC, texture of RBC and location of the chromatin) to be used in their classification protocol. One of them proposed the detection system consisting of six stages: nucleated components detection, components detection, image decomposition, erythrocyte size estimation, leukocytes and malaria gametocytes classification, and parasitemia estimation.



a) b) c) d)

Figure 1. a) Plasmodium Falciparum (b) P. Vivax (c) P. Malariae (d) P. Ovale

Malaria is classified into either "severe" or "uncomplicated" by the World Health Organization (WHO). It is deemed severe when any of the following criteria are present, otherwise it is considered uncomplicated.

- Decreased consciousness
- Significant weakness such that the person is unable to walk
- Inability to feed
- Two or more convulsions
- Low blood pressure (less than 70 mmHg in adults and 50 mmHg in children)
- Breathing problems
- Circulatory shock
- Kidney failure or hemoglobin in the urine etc

Diagnosis can be difficult where malaria is no longer endemic for healthcare providers unfamiliar with the disease. Clinicians may forget to consider malaria among the potential diagnoses for some patients and not order the necessary diagnostic tests. Technicians may be unfamiliar with, or lack experience with, malaria, and fail to detect parasites when examining blood smears under a microscope. In some areas, malaria transmission is so intense that a large proportion of the population is infected but remains asymptomatic

Figure 2 below shows fully automated image classification system to positively identify malaria parasites present in thin blood smears, and differentiate the species.

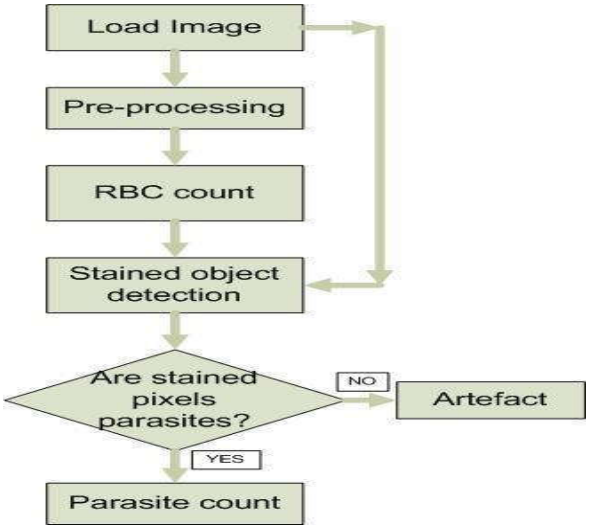


Figure 2 Fully-automated image classification system

The first step is to acquire the images of malaria samples. In this study, the malaria images of ring, trophozoite and gametocyte stages have been captured from the thin blood smears of *P. vivax* samples. The malaria slides are prepared by Medical Microbiology & Parasitology Department, Hospital University Science Malaysia (HUSM). Each slide has been stained by using the Giemsa staining. The malaria slides are examined using 100X oil immersion objective of Leica DLMA microscope. The images are then captured using Infinity-2 digital camera at a resolution setting of 800×600 pixels and saved in BMP format. The captured images are studied under the supervision of microbiologists in order to recognize and differentiate between the three life-cycle stages of *P. vivax* species. The captured images are shown in figure 2.

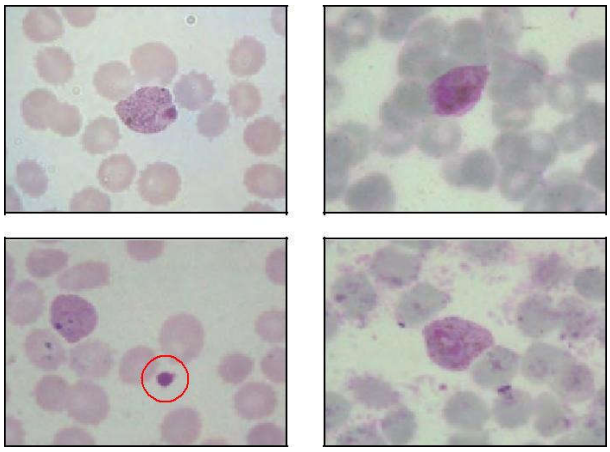


Figure 3 Samples of the captured malaria images

The purpose of pre-processing is to remove unwanted objects and noise from the image to facilitate image segmentation into meaningful regions. The steps required to carry out image pre-processing were implemented on low resolution images are as follows

- Load coloured (RGB) or gray scale image, the coloured image is converted to gray scale image. The contrast of the gray scale image is enhanced using local histogram equalization to enhance the visibility of the parasites and RBC.
- distinguish objects from background. The common way

described in the literature is to use edge detection algorithms. Edge detection or boundary detection algorithm use to segment image into meaningful regions, i.e. RBC and artefacts from the background is shown in Figure 4

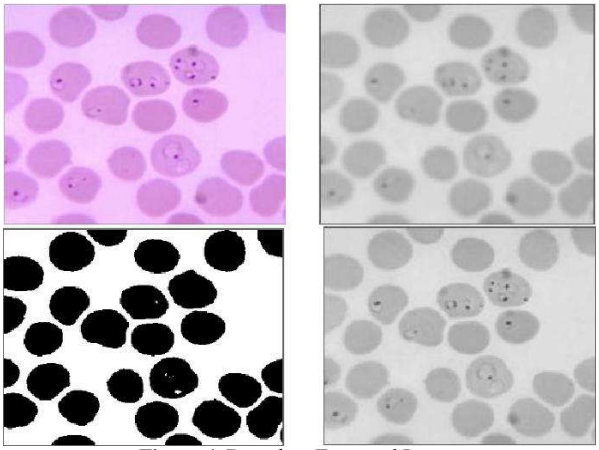


Figure 4 Boundary Extracted Image

Current malaria diagnosis relies primarily on microscopic examination of Giemsa-stained thick and thin blood films. This method requires vigorously trained technicians to efficiently detect and classify the malaria parasite species such as Plasmodium falciparum (Pf) and Plasmodium vivax (Pv) for an appropriate drug administration. However, accurate classification of parasite species is difficult to achieve because of inherent technical limitations and human inconsistency. To improve performance of malaria parasite classification, many researchers have proposed automated malaria detection devices using digital image analysis. These image processing tools, however, focus on detection of parasites on thin blood films, which may not detect the existence of parasites due to the parasite scarcity on the thin blood film. The problem is aggravated with low parasitemia condition. Automated detection and classification of parasites on thick blood films, which contain more numbers of parasites per detection area, would address the previous limitation.

Differentiation of clinical diagnoses from other tropical infections, based on patients' signs and symptoms or physicians' findings, may be difficult. Therefore, confirmatory diagnoses using laboratory technologies are urgently needed. This review discusses on the currently available diagnostic methods for malaria in many settings, and assesses their feasibility in resource-rich and resource-poor settings.

DIFFERENT METHODS

The definitive diagnosis of malaria infection is done by searching for parasites in blood slides (films) through a microscope. In peripheral blood sample visual detection and recognition of Plasmodium spp is possible and efficient via a chemical process called (Giemsa) staining. The staining process slightly colorizes the red blood cells (RBCs) but highlights Plasmodium spp parasites, white blood cells (WBC), and platelets or artifacts. The detection of Plasmodium spp requires detection of the stained objects. However, to prevent false diagnosis the stained objects have to be analyzed further to determine if they are parasites or not. Figure 5 shows malaria effected blood images.

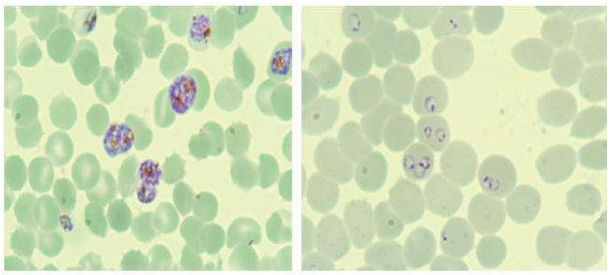


Figure 5 Malaria Effected Blood Images

A clinical diagnosis of malaria is traditional among medical doctors. This method is least expensive and most widely practiced. Clinical diagnosis is based on the patients' signs and symptoms, and on physical findings at examination. The earliest symptoms of malaria are very nonspecific and variable, and include fever, headache, weakness, myalgia, chills, dizziness, abdominal pain, diarrhea, nausea, vomiting, anorexia, and pruritus[8]. A clinical diagnosis of malaria is still challenging because of the non-specific nature of the signs and symptoms, which overlap considerably with other common, as well as potentially life-threatening diseases. Technicians may be unfamiliar with, or lack experience with, malaria, and fail to detect parasites when examining blood smears under a microscope. Therefore, the accuracy of malaria diagnosis can be greatly enhanced by combining clinical-and parasite-based findings.

In the laboratory, malaria is diagnosed using different techniques, e.g. conventional microscopic diagnosis by staining thin and thick peripheral blood smears [9], other concentration techniques, e.g. quantitative buffy coat (QBC) method [8], rapid diagnostic tests e.g., OptiMAL [10,11], ICT [12], Para-HIT-f [13], ParaScreen [14], SD Bioline [15], Paracheck [16], and molecular diagnostic methods, such as polymerase chain reaction (PCR) [17-18].

Malaria is conventionally diagnosed by microscopic examination of stained blood films using Giemsa, Wright's, or Field's stains [19]. This method has changed very little since Laverran's original discovery of the malaria parasite, and improvements in staining techniques by Romanowsky in the late 1,800s. More than a century later, microscopic detection and identification of *Plasmodium* species in Giemsa-stained thick blood films (for screening the presenting malaria parasite), and thin blood films (for species' confirmation) remains the gold standard for laboratory diagnosis [20].Malaria is diagnosed microscopically by staining thick and thin blood films on a glass slide, to visualize malaria parasites.

The wide acceptance of this technique by laboratories all around the world can be attributed to its simplicity, low cost, its ability to identify the presence of parasites, the infecting species, and assess parasite density-all parameters useful for the management of malaria. However, the staining and interpretation processes are labor intensive, time consuming, and require considerable expertise and trained healthcare workers, particularly for identifying species accurately at low parasitemia or in mixed malarial infections. The most important shortcoming of microscopic examination is its relatively low sensitivity, particularly at low parasite levels.

The QBC technique was designed to enhance microscopic detection of parasites and simplify malaria diagnosis. This method involves staining parasite deoxyribonucleic acid (DNA) in micro-hematocrit tubes with fluorescent dyes, e.g. acridine orange, and its subsequent detection by epi-fluorescent microscopy. Briefly, finger-prick blood is collected in a hematocrit tube containing acridine orange and anticoagulant. The tube is centrifuged at 12,000 g for 5 min and immediately examined using an epi-fluorescent microscope. Parasite nuclei fluoresces bright green, while cytoplasm appears yellow-orange. The QBC technique has been shown to be a rapid and sensitive test for diagnosing malaria in numerous laboratories settings. While it enhances sensitivity for *P. falciparum*, it reduces sensitivity for non-falciparum species and decreases specificity due to staining of leukocyte DNA. QBC technique is simple, reliable, and user-friendly, it requires specialized instrumentation, is more costly than conventional light microscopy, and is poor at determining species and numbers of parasites.

Rapid diagnostic test is a device that detects malaria antigen in a small amount of blood, usually 5–15 μL, by immunochromatographic assay with monoclonal antibodies directed against the target parasite antigen and impregnated on a test strip. The result, usually a colored test line, is obtained in 5–20 min. RDTs require no capital investment or electricity, are simple to perform, and are easy to interpret.

RDTs for malaria are fast and easy to perform, and do not require electricity or specific equipment. Unlike conventional microscopic diagnosis by staining thin and thick peripheral blood smears, and QBC technique, RDTs are all based on the same principle and detect malaria antigen in blood flowing along a membrane containing specific anti-malaria antibodies. Most products target a *P. falciparum*-specific protein, e.g. histidine-rich protein II (HRP-II) or lactate dehydrogenase (LDH). Some tests detect *P. falciparum* specific and pan-specific antigens (aldolase or pan-malaria pLDH), and distinguish non-*P. falciparum* infections from mixed malaria infections. Although most RDT products are suitable for *P. falciparum* malaria diagnosis, some also claim that they can effectively and rapidly diagnose *P. vivax* malaria.

In the last decade, there has been an upsurge of interest in developing malaria rapid diagnostic test (RDT) kits for the detection of Plasmodium species. Three antigens - Plasmodium falciparum histidine-rich protein 2 (PfHRP2), plasmodial aldolase and plasmodial lactate dehydrogenase (pLDH) - are currently used for RDTs. Tests targeting HRP2 contribute to more than 90% of the malaria RDTs in current use. However, the specificities, sensitivities, numbers of false positives, numbers of false negatives and temperature tolerances of these tests vary considerably, illustrating the difficulties and challenges facing current RDTs.

Overall, RDTs appears a highly valuable, rapid malaria-diagnostic tool for healthcare workers; however it must currently be used in conjunction with other methods to confirm the results, characterize infection, and monitor treatment. In malaria-endemic areas where no light microscopy facility exists that may benefit from RDTs, improvements are required for ease of use, sensitivity for non-falciparum infection, stability, and affordability. The WHO is now developing guidelines to ensure lot-to-lot quality control, which is essential for the community's confidence in this new diagnostic tool. Because the simplicity and reliability of RDTs have been improved for use in rural endemic areas, RDT diagnosis in non-endemic regions is becoming more feasible, which may reduce time-to-treatment for cases of imported malaria. Most RDTs today have achieved this goal for P. falciparum, but not for non-P. falciparum.

Diagnosis of malaria using serological methods is usually based on the detection of antibodies against asexual blood stage malaria parasites. Immunofluorescence antibody testing (IFA) has been a reliable serologic test for malaria in recent decades.IFA is simple and sensitive, but time-consuming. It cannot be automated, which limits the number of sera that can be studied daily. It also requires fluorescence microscopy and trained technicians; readings can be influenced by the level of training of the technician, particularly for serum samples with low antibody titers.

Recent developments in molecular biological technologies, e.g. PCR, loop-mediated isothermal amplification (LAMP), microarray, mass spectrometry (MS), and flow cytometric (FCM) assay techniques, have permitted extensive characterization of the malaria parasite and are generating new strategies for malaria diagnosis.

Polymerase chain reaction (PCR) detects parasite DNA, can identify infections below the threshold of detection for microscopy and RDTs, and is commonly considered the gold standard to detect malaria infection. However, PCR requires sophisticated laboratory infrastructure and advanced training, making it challenging and costly to implement in most malaria-endemic areas. Molecular methods, namely, DNA probes and PCR were introduced in the 1980s–1990s. PCR-based techniques are a recent development in the molecular diagnosis of malaria, and have proven to be one of the most specific and sensitive diagnostic methods, particularly for malaria cases with low parasitemia or mixed infection. Concerning with the gold standard method for malaria diagnosis, PCR has shown higher sensitivity and specificity than conventional microscopic examination of stained peripheral blood smears, and now seems the best method for malaria diagnosis. PCR appears to have overcome the two major problems of malaria diagnosis-sensitivity and specificity- the utility of PCR is limited by complex methodologies, high cost, and the need for specially trained technicians.

Loop-mediated isothermal amplification (LAMP) may offer a practical alternative. LAMP is more reliable and useful for routine screening for malaria parasites in regions where vector-borne diseases, such as malaria, are endemic. LAMP is a technique that enables DNA amplification with high specificity, sensitivity, and rapidity under isothermal conditions. DNA amplification can be achieved using simple incubators (water bath or block heater) because of these isothermal conditions. LAMP appears to be easy, sensitive, quick and lower in cost than PCR. However, reagents require cold storage, and further clinical trials are needed to validate the feasibility and clinical utility of LAMP.

CONCLUSIONS

Conventional microscopic examination of peripheral thick and

thin blood smears remains the gold standard for malaria diagnosis.Quick and convenient RDTs are currently implemented in many remote settings, but are costly and need improved quality control. Serological tests are useful for epidemiological surveys, but not suitable for the diagnosis of acute malaria. Molecular-biological techniques are appropriate for research laboratories; they can be used to identify the development of drug-resistance, are useful for species identification, and also for quantifying parasite density with low parasitemia. This paper reviews some of the well known methods for malaria diagnosis.

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