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Advance techniques for diagnosis of malaria: A review

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Abstract
Malaria is a major cause of death in tropical and sub-tropical countries, killing each year over 1 million people globally; 90% of fatalities occur in African children. Although effective ways to manage malaria now exist, the number of malaria cases is still increasing, due to several factors. In this emergency situation, prompt and effective diagnostic methods are essential for the management and control of malaria. Traditional methods for diagnosing malaria remain problematic; therefore, new technologies have been developed and introduced to overcome the limitations. This review details the currently available diagnostic methods for malaria.

Keywords: Malaria, PCR, RDT

Introduction

Malaria is a mosquito-borne disease caused by a parasite. People with malaria often experience fever, chills, and flu-like illness. If left untreated, they may develop severe complications and die [1].

Four *Plasmodium species* are responsible for human malaria these are *P. falciparum, P. vivax, P. ovale and P. malariae* [2]. In 2007, after returning from Malaysia, a Finnish tourist was found to be infected with a fifth *Plasmodium species*, *P. knowlesi* which usually infects macaques. Over the past few years, hundreds of human cases have been found in Malaysia. The clinical disease caused by *P. knowlesi* appears less severe than *P. falciparum* infection, but more severe than infection with other malaria-causing species. Diagnosis is based both on PCR and microscopy. *P. knowlesi* is currently considered as the fifth species causing malaria in humans [3].

The World Health Organization estimates that half the world's population are at risk of malaria, with 225 million people developing clinical malaria in 2009 (78% in Africa), and 781,000 deaths (91% in Africa, most being children). Malaria remains endemic in 106 countries, and while parasite-based diagnosis is increasing, most suspected cases of malaria are still not properly identified, resulting in over-use of anti malarial drugs and poor disease monitoring [4]. The traditional method of microscopic identification of malaria is time consuming, daunting in poor power setting and requiring a lot of expertise/training [5]. So some new techniques have been developed in diagnosis of malaria.

Advance techniques in rapid diagnosis of malaria
Fluorescent Microscopy

It uses readily-prepared and ready to-use slides labelled with an unspecific DNA-binding fluorescent dye (4'-6-Diamidino-2-phenylindole (DAPI); emission 443 nm) that detects *Plasmodium* DNA. Sample is placed on dye labelled area of a slide, cover slipped, incubated at room temperature for a minute and observed under the 40× objective under UV light (365 nm). The presence of bright shiny tiny dots under the UV light indicates the presence of malarial parasites (figure 2). To prevent the slides from drying out, they are kept in a wet chamber. Positive and negative controls are done for each batch of test kits [6].

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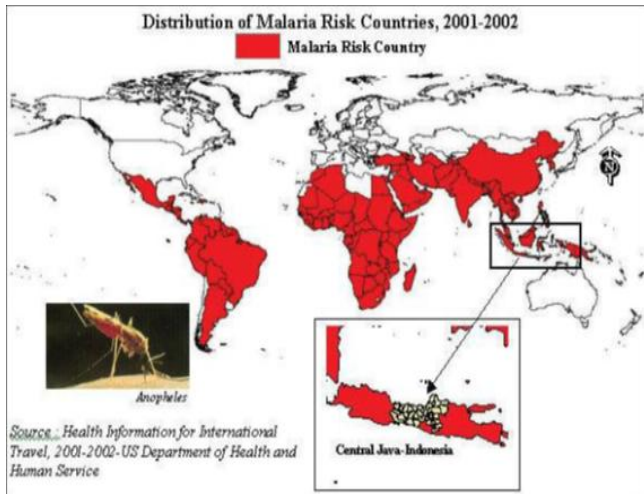MM Institute of Nursing,
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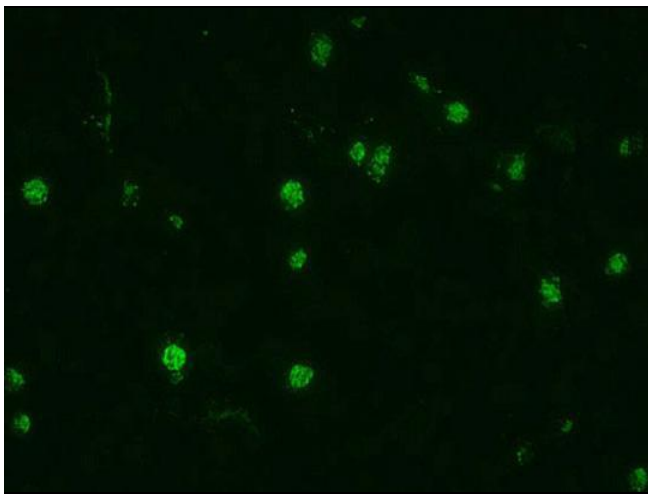**Fig 1:** The incidence rate of Malaria worldwide

Fig 2: Positive Blood Smear under Fluorescent Microscope

Quantitative Buffy Coat (Qbc) Technique

The speed of QBC method (15 min) in detecting malarial parasites is a definite advantage in laboratories which screen large number of samples. In addition, low levels of parasitaemia (2 parasites/µl) can easily be detected as more blood is being used per sample (55-65µl). There is no loss of parasites during the procedure. The parasitized erythrocytes are concentrated in the small area of buffy coat, which helps in rapid scanning of the parasite. The QBC tube is placed on the tube holder and examined using a standard white light microscope equipped with the UV microscope adapter, an epi-illuminated microscope objective. Fluorescing parasites are then observed at the red blood cell/white blood cell interface [7].

PCR (Polymerase Chain Reaction)

Analysis of DNA by the polymerase chain reaction (PCR) can be a useful tool for diagnosis of malaria when the results of conventional techniques are negative, especially since PCR allows accurate species identification and can detect low level parasitaemia. PCR has a sensitivity and specificity of 100 percent with a detection limit of just one *P. falciparum* or three *P. vivax* parasites per micro litre of blood when compared with the gold standard. PCR has also been credited to have been able to detect mixed infections with ease in many studies [8]. Several molecular methods based on the amplification of DNA have been developed for the detection of malarial infection in humans i.e. semi-nested multiplex malaria PCR (SnM-PCR) and real time quantitative PCR (qPCR) [9].

Semi-nested-PCR detects *Plasmodium species* DNA *(P. vivax, P. Falciparum* and *P. malariae)* by amplification of the small sub-unit ribosomal ribonucleic acid (ssrRNA) genes using the primers and cycling parameters. This assay could detect the presence of one to ten parasites/µl of blood [10].

Real-time PCR assays targeting *Plasmodium falciparum* lactate dehydrogenase (pfldh) gene may facilitate the identification of a high proportion of pregnant women with a *P. falciparum* parasitaemia below the threshold of microscopy [11].

Immunochromatographic Test

This test is based on the capture of the parasite antigens from the peripheral blood using either monoclonal or polyclonal antibodies against the target antigen [12]. This test can detect malaria antigen in a small amount of blood, usually 5–15 µl, by immunochromatographic assay [13].

RDTs (Rapid Diagnostic Tests) consist of nitrocellulose strips mostly embedded in plastic cassettes. When blood and buffer are applied, the red blood cells are lysed and the targeted antigen binds to the detecting mouse antibody which is conjugated to colloidal gold. This complex moves further along the nitrocellulose strip until the antigen binds to the capture antibody embedded as a transverse line on the nitrocellulose strip. As a result, the colloidal gold is concentrated on a small surface and becomes visible as a purple-red line. The non-bound conjugated antibodies move further along the strip until they are captured by goat anti-mouse antibodies, thereby generating the control line. Two-band RDTs consist of a control line and a *P. falciparum* specific test line which targets either histidine-rich protein-2 (HRP-2) or *P. falciparum* specific lactate dehydrogenase (Pf-pLDH). Three-band RDTs display three lines: a control line, a *P. falciparum*-specific line (detection of HRP-2 or Pf-pLDH) a third line detecting *P. vivax* (by a *P. vivax*- specific pLDH, Pv-pLDH) or an antigen common to all four species, either aldolase or pan-*Plasmodium*-specific pLDH (pan-pLDH) [14].
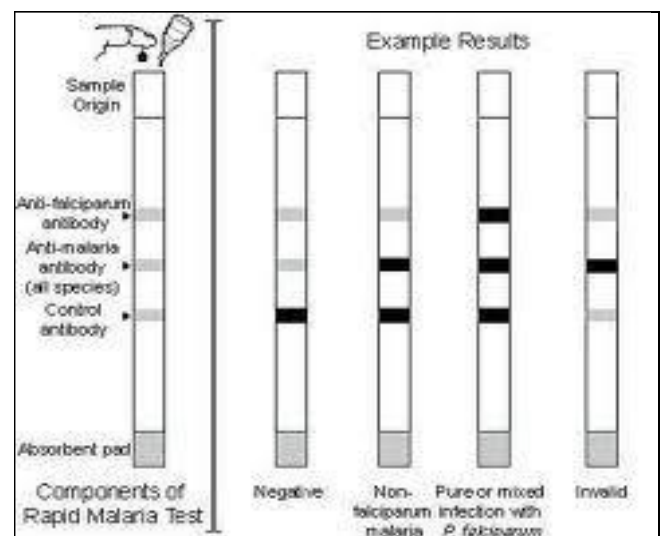

Fig 3: Interpretation of Rapid Diagnostic Test (RDT)

Laser Desorption Mass Spectrometry (Ldms)

This method is based on the detection of heme in hemozoin (Hz), the crystalline substance accumulated within malaria

parasites during their intraerythrocytic growth stage. The LDMS test requires no consumables other than a lancet and a container for blood collection. Blood is diluted in water, deposited onto a metal slide, air dried, and then inserted into the mass spectrometer for analysis. Hz heme is identified from the pattern of heme molecular structure-specific peaks. A correlation filter (CF) algorithm is used to score local mass spectra for the presence of Hz heme during spatial scanning of the laser beam across the sample [15, 16].

Conclusion
We concluded that the advance method for detection of Malaria has many advantages over old method. RDTs kits are most commonly used in diagnosis of malaria as these are cost effective, easily available, very fast results and no trained technician needed.

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