Laboratory diagnosis of malaria infection – A short review of methods

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Abstract
Malaria is one of the most important tropical infectious diseases. The incidence of malaria worldwide is estimated to be 300–500 million clinical cases each year with a mortality of between one and three million people worldwide annually. The accurate and timely diagnosis of malaria infection is essential if severe complications and mortality are to be reduced by early specific antimalarial treatment. This review details the methods for the laboratory diagnosis of malaria infection.

Keywords: malaria, diagnosis, microscopy, rapid

Introduction
Malaria is the most important parasitic infection of man, and is associated with a huge burden of morbidity and mortality in many parts of the tropical world. Mortality rates of 10–30% have been reported among children referred to hospitals with severe malaria, although these rates are even higher in rural and remote areas where diagnosis and treatment are not readily available (1). The accurate diagnosis of malaria infection is important in order to reduce severe complications and mortality. Malaria infection cannot be diagnosed clinically as the presenting clinical signs and symptoms mimic other tropical infections and therefore must be confirmed by laboratory diagnosis. This review focuses on the different laboratory tools available for confirmation of malaria infection. It should be noted, however, that although in non-immune individuals the presence of malaria parasites in the blood is sufficient to make a diagnosis of clinical malaria infection, in immune individuals living in high transmission areas there are high rates of asymptomatic parasitaemia; in this context alternative causes of the presenting illness need to be considered as the parasitaemia may be incidental.

The simple, direct microscopic observation of blood specimens to observe the malaria parasite is still the gold standard for malaria diagnosis. Microscopic diagnosis of malaria is performed by staining thick and thin blood films on a glass slide to visualize the malaria parasite (2). Briefly, the patient's finger is cleaned with alcohol, allowed to dry and then the side of the fingertip is pricked with a sharp sterile lancet or needle and two drops of blood are placed on a glass slide. To prepare a thick blood film, a blood spot is stirred in a circular motion with the corner of the slide, taking care not to make the preparation too thick, and allowed to dry without fixative. As they are unfixed the red cells lyse when a water based stain is applied. A thin blood film is prepared by immediately placing the smooth edge of a spreader slide in the drop of blood, adjusting the angle between slide and spreader to 45°, and then smearing the blood with a swift and steady sweep along the surface. The film is then allowed to air dry and is fixed with methanol. Because a larger volume of blood is examined the thick film is more sensitive than the thin film (down to around 40 parasites per µL or 1 parasite per 200 white blood cells) although it requires more expertise to read (3).

Alternatively, an intradermal smear may be prepared by making multiple intradermal punctures with a 25 gauge needle on the surface of the upper forearm. The punctures should not ooze blood, but serosanguinous fluid; this is expressed on the glass by squeezing and smeared and allowed to air dry and fixed with methanol. This smear may show pigment-containing leucocytes and demonstrate more mature forms of Plasmodium falciparum than peripheral blood (4). There are many methods described for staining blood films for malaria diagnosis, including Giemsa stain (20-30 min), Leishman stain (45 min), and the rapid Field stain method (10 sec) (5). On light microscopic examination of the blood film the number, species, and morphological stage of the parasites can be reported (Figure 1). Sometimes parasites cannot be found in peripheral blood smear from patients with malaria, but malaria pigment may be seen in circulating phagocytic leucocytes. This is a pathognomonic sign of recent malaria infection, and in the case of a treated or partially treated infection may be seen in the absence of parasites. The presence of leucocyte pigment is both qualitatively and quantitatively associated with parasite load, and is therefore indicative of a clinically significant infection, particularly in low transmission areas (6). Parasites may also be found in bone marrow aspirate when absent...
Figure 1. Left: photomicrographs of stage development (ring, trophozoite and schizont) of P. falciparum, P. vivax, P. malariae and P. ovale from thin blood smears.

Right: photomicrographs of P. falciparum from thin blood smear (top), thick blood smear (middle) and brain smear (bottom)
on thin smears of the peripheral blood (7).

In addition to providing a diagnosis of malaria the blood smear can also provide useful prognostic information; the parasite count, number of circulating pigment-containing phagocytes and the presence of late asexual stages of the parasite are all positively correlated with a fatal outcome (4,6).

**Molecular methods**

The polymerase chain reaction (PCR) allows the specific amplification of a selected region of the malarial genome (8). This technique is highly specific and sensitive (1-5 parasite/mL of blood) and permits genotyping (9,10). Furthermore, PCR using single nucleotide polymorphism (SNP) analysis allows the detection of drug resistant parasites and mixed infections (11,12) However, PCR is expensive and requires a sophisticated laboratory manned with well-trained staff.

**Rapid methods**

Detection in patient samples of malaria parasite antigens such as histidine rich protein II (HRP-II) or plasmodium lactate dehydrogenase (pLDH) can be performed by rapid, point-of-care tests based on immunochromatographic methods.

There are many commercially-available rapid tests (see Table 1 for summary) including Para Sight F (13,14) and Paracheck, Binax NOW P/I /Fv. and OptiMAL (Flow Inc., USA) (15,16).

Excellent reviews of the diagnostic performance of rapid methods for the diagnosis of malaria are presented elsewhere (17,18). The advantages of these tests are that they are quick to perform and have high sensitivity (19). The disadvantages of the rapid format are the relatively high cost, the inability of some tests to distinguish malarial species, and manufacturing variation (18). Those based on HRP II detection may give positive results in the convalescent phase of the illness due to the persistence of HRP II in the blood after parasite clearance (20).

**Quantitative buffy coat method**

Quantitative buffy coat (QBC; Becton Dickinson, USA) is a method for identifying the malarial parasite in the peripheral blood. It involves staining of the centrifuged and compressed red cell layer with acridine orange and its examination under an ultraviolet (UV) light source (21). Briefly, blood is collected (from a finger prick) in an haematocrit tube containing acridine orange and anticoagulant. The haematocrit tube is centrifuged at 12,000 g for 5 min and immediately examined using a microscope equipped with a UV light source.

The parasite nuclei fluoresce bright green, and the cytoplasm appears yellow-orange. This test has sensitivity similar to the conventional thick blood film microscopic methods. It is reliable and user-friendly and should be used together with thick blood film microscopic screening. However, QBC requires specialised instrumentation, has a higher high cost than microscopic methods and is poor at species determination and parasite quantification.

**Serological methods**

Serological tests for the diagnosis of malaria infection rely on the detection of antibodies against asexual blood stages of the malaria parasite. The first serological test used for the detection of malaria antibodies was the immunofluorescence assay, often abbreviated to IFA (22). This method uses specific antigen or crude antigen prepared on a slide, coated and kept at ~30 °C until use, and quantifies both IgG and IgM antibodies in patient serum samples. Titres >1.20 are classified as positive, and those below 1.20 classified as of doubtful significance. High titres (>1.200) represent strong evidence of a recent infection. Serological tests provide retrospective confirmation of malaria infection or a history of infection, and are useful in epidemiology surveys and the screening of blood collected for blood banks. Nevertheless, the utility of serological methods for the diagnosis of acute malaria infection is limited owing to the delay in antibodies development, lack of species confirmation and the need for a fluorescence (UV) microscope.

Other malaria diagnostic methods include the cultivation of live malaria parasites (23,24), and post-mortem diagnosis made by the detection of malaria parasites or pigment in leucocytes in tissue autopsy, brain smears (25), spleen imprint and bone narrow smear.

**Conclusions**

The diagnosis of malaria by conventional microscopy remains the gold standard for malaria diagnosis, although it requires highly-skilled personnel and may have a lower sensitivity than the more recent molecular techniques. It is, however, inexpensive and reliable. Rapid assays are expensive but are quick and convenient. Molecular techniques are better suited to research laboratories to check for development of drug resistance and relapse, and can be useful for species identification when counts are very low or samples have undergone some deterioration. Serology is best used as an epidemiological tool and is not suitable for the acute diagnosis of malaria. The choice of the most appropriate test for malarial diagnosis must be determined by the level of malarial endemity (including species), the urgency of diagnosis, and availability of personnel and financial resources.

**Acknowledgement**

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**References**

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Table 1. Description of rapid assays for the diagnosis of malaria infection.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Manufacturer</th>
<th>Antigen</th>
<th>Species specificity</th>
</tr>
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<tbody>
<tr>
<td>Para Sight F</td>
<td>Becton-Dickenson, USA</td>
<td>HRP-II*</td>
<td>P. falciparum</td>
</tr>
<tr>
<td>Paracheck</td>
<td>Orchid Biochemicals, India</td>
<td>HRP-II</td>
<td>P. falciparum</td>
</tr>
<tr>
<td>NOW P.f/Pv</td>
<td>Binax Inc, USA</td>
<td>HRP-II</td>
<td>P. falciparum, P. vivax, P. malariae, P. ovale</td>
</tr>
<tr>
<td>OptiMAL</td>
<td>Flow Inc, USA</td>
<td>pLDH†</td>
<td>P. falciparum, P. vivax, P. malariae, P. ovale</td>
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* Histidine Rich Protein II
† Lactate Dehydrogenase