

## MALARIA: SMEARS OR BUFFY COATS?

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Malaria in humans is caused by one of four protozoan species of the genus *Plasmodium*: *P falciparum*, *P vivax*, *P ovale*, and *P malariae*. All are transmitted by the bite of an infected female *Anopheles* mosquito. The disease is estimated to have a worldwide prevalence in excess of 100 million cases associated with approximately 1 million deaths per year in Africa alone<sup>(1,2)</sup>. Transmission occurs in large areas of Central and South America, Africa, India, Southeast Asia, the Middle East, and Oceania<sup>(3)</sup>. The disease continues to threaten even countries thought to have controlled or eliminated it. An increase in the number of imported cases in these countries, such as the United States, has been associated with a rise in international travel. Malaria caused by multi-drug-resistant *P falciparum* is a major cause of morbidity and mortality in tropical countries, particularly in the malarious areas of Southeast Asia<sup>(4,5)</sup>. Therefore, improving early diagnosis and treatment has been a major thrust of malaria control programmes and rapid and accurate methods of diagnosis are especially important for cases of severe and complicated malaria.

Present methods for the diagnosis of malaria range from the very simple to the very complex<sup>(6)</sup>. The simple microscopic diagnosis of malaria continues to be an indispensable tool both in clinical practice and in epidemiological investigations. Serological methods, which include the indirect fluorescent antibody technique and the enzyme-linked-immunosorbent assay, are of limited value for the diagnosis of acute malaria<sup>(6)</sup>. However, they are useful for screening asymptomatic potential blood donors who may have low-level parasitaemia and in the detection of otherwise occult infections. Serological tests have also been employed in epidemiological studies of the prevalence of malaria, trends of transmission, and are particularly useful in field studies of malaria vaccines. The molecular approach to the detection of malaria by deoxyribonucleic acid probes<sup>(7)</sup> is complex. Although the sensitivity of these probes is comparable with that of microscopy, these probes need to be simplified for wider usage<sup>(8)</sup>. A simple system for the sensitive and accurate diagnosis of all four species of the malaria parasite based on ribonucleic acid hybridisation techniques has also been developed but its wide application remains to be seen<sup>(9)</sup>.

The diagnosis of malaria rests on the demonstration of parasites in stained peripheral blood smears, a technique which has little improved since its introduction in 1903<sup>(6,8,10)</sup>. This method is highly sensitive and is species- and stage-specific in experienced hands. However, as symptoms of malaria may precede detectable parasitaemia by a few days, a diagnosis of malaria can only be excluded by obtaining negative blood

smears on several successive days. Therefore, a single blood smear examination does not exclude the diagnosis of malaria. The blood film methods are widely used for the diagnosis of malaria, both in the laboratory and the field. Thick smears, which have the advantage of allowing the examination of a larger volume of blood than is possible using thin smears, improve the chance of detection of plasmodia by a factor of 20<sup>(6)</sup>. In thick film preparations, the concentrated erythrocytes are lysed with distilled water, revealing intact parasites. The thick smear is only useful for the detection of the presence of parasites and quantitating parasitaemia, but not for species diagnosis. In contrast, the thin film can be used for detection of parasites as well as species diagnosis. While the Giemsa stain has been commonly used to stain the blood film preparations, Field's stain has also been employed for its speed and simplicity<sup>(11)</sup>.

Light microscopy allows rapid estimation of the level of parasitaemia and an assessment of the degree of concomitant anaemia within about 20 minutes, including staining time. However, the detection of plasmodia on blood smears is very much dependent on the skill and training of the microscopist, the staining techniques, the time taken in screening the films, the quality of equipment used, and work and environmental conditions. Smear sensitivity has been reported to be directly proportional to the time taken to screen, and at least 100 oil immersion microscopic fields should be viewed<sup>(12)</sup>. Artifacts are numerous in thick smears and correct interpretation requires experience. In view of these, and also to reduce the dependence on highly skilled laboratory staff and equipment, new approaches for malaria detection have been explored by researchers, especially for epidemiological studies and mass surveys.

In 1983, Wardlaw and Levine<sup>(13)</sup> developed a rapid system for the quantitative analysis of the buffy coat in centrifuged whole blood samples. The method was called the quantitative buffy coat (QBC) analysis. A modified microhaematocrit tube precoated with acridine orange and containing a plastic float (the QBC tube) was used as a novel means of physically expanding and separating the buffy coat into three distinct layers that consist of granulocytes, non-granulocytes (lymphocytes and monocytes) and platelets. The QBC analysis was able to provide a haematocrit value and leucocyte quantification. Malaria-infected erythrocytes which are lighter than non-infected cells but somewhat heavier than granulocytes are concentrated in the uppermost layer of the red cell pack. These infected cells are stained with acridine orange and can be detected by direct inspection of ultraviolet-illuminated tubes. As malaria and other parasites were detected in preliminary studies using animal models, the QBC analysis was suggested as a useful diagnostic tool in studies of parasitic diseases.

Subsequently, various studies were conducted using the method for the diagnosis of malaria in humans<sup>(14-16)</sup>. Spielman et al<sup>(14)</sup> applied the method on subjects suspected to have malaria in Ethiopia, and described the QBC technique as rapid, sensitive and specific enough for use under field conditions. The method was reported to be eight times more sensitive than conventional light microscopy and about 10% more malaria

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infections were recognised. However, this increase in sensitivity could not be demonstrated in the field by Pornsilapatip et al<sup>(15)</sup>. The method was described as ideal for use in the field as the materials used did not require refrigeration and may be stored for a long time. Rickman et al<sup>(16)</sup> reported a sensitivity of 70% and a specificity of 98.4% when the QBC technique was used for mass screening in the field, and that its speed and ease of use make it an important new tool for the diagnosis of malaria. Processing and interpretation of the QBC tube were said to be easier and much more rapid than that for a thick blood film, with only ten minutes spent for both centrifugation and examination. In addition, the diagnosis of malaria may be facilitated even by inexperienced microscopists. However, speciation using this method was poor compared with microscopy. While high sensitivity and specificity in excess of 90% were reported under field conditions using the QBC tube in Thailand, speciation was less than 80%<sup>(15)</sup>. Wongsrichanalai et al<sup>(17)</sup> conducted a survey in villages endemic for malaria in Thailand but reported that the sensitivity of the test was less than 80% and that speciation was imperfect. In a recent field survey conducted in Malaysia, the researchers acknowledged the rapidity of the QBC technique, but reported that sensitivity and speciation was less than 60%<sup>(18)</sup>. Pornsilapatip et al<sup>(15)</sup> noted the high sensitivity of the test at parasite densities of 100 per  $\mu$ l or lower and that the QBC tube could detect plasmodia as low as one parasite per  $\mu$ l. However, other studies could only demonstrate high sensitivity at parasite densities of 100 per  $\mu$ l and above<sup>(16,18)</sup>.

While the use of the QBC tube as a rapid method of diagnosis of malaria is promising, its adoption for use in the rural settings is still controversial and has limitations as the technique requires a microcentrifuge and ultraviolet microscopy. Studies indicated that the validity of the method was inconsistent and that the species of parasite were only correctly identified in less than 80% of cases using this method<sup>(14,18)</sup>. Rickman et al<sup>(16)</sup> cautioned that the QBC tube cannot substitute the blood smear for species identification. White and Silamut<sup>(11)</sup> commented that although the acridine orange technique may be of value in research and for mass screening, it will not replace the well-established light microscopic examination in the diagnosis of malaria.

The applicability and acceptability of the QBC analysis as a newer method of rapid diagnosis for use widely within malaria endemic regions remain to be seen. While high sensitivity will be important for a test that is to be used for patient diagnosis in areas with relatively low endemicity, it will not be crucial in areas with intensive malaria transmission<sup>(8)</sup>. How-

ever, for epidemiological studies, a test with high sensitivity and specificity is often necessary. More studies would be needed to assess the validity of the QBC technique in different situations. Meanwhile, sacrificing the sensitivity and specificity of a well established procedure for the rapidity of a newer test with inconsistent validity is certainly unwise in a disease which is potentially fatal. The microscopic examination of blood smears currently remains the most sensitive and reliable method for the detection and identification of malaria parasites. The continuous quest for newer and better techniques for the rapid diagnosis of malaria is evident in a recent report<sup>(19)</sup> that malarial antigen detection by monoclonal antibody is an extremely rapid and sensitive method which could be adopted for use in endemic countries.

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