Screening for malaria: the QBC technique

Prompt laboratory diagnosis of malaria is crucial for effective disease management. The standard diagnostic method is the microscopic examination of Giemsa-stained blood films. The Quantative Buffy Coat (QBC) is an Acridine Orange fluorescence staining method, used as a preliminary screening tool for the diagnosis of malaria. This fast, inexpensive technique is more sensitive than the examination of Giemsa-stained slides.

by J. Gallagher

Malaria is an infectious disease that is widespread in the tropical and subtropical areas of Asia, Africa and the Americas. Approximately 515 million cases are diagnosed per year and in Africa, one to three million of these cases are fatal. The disease is caused by the transmission of protozoan parasites of the genus *Plasmodium*, via the female Anopheles mosquito. In humans, malaria is caused by *P. falciparum, P. vivax, P.ovale* and *P. malariae*, and incidentally by *P. knowlesi*, a zoonotic infection which can be transmitted to humans from the macaque monkey via the mosquito *Anopheles latens*. *P. falciparum* is the most common cause of the disease and is responsible for about 80% of all malaria cases and 90% of deaths from the disease. Within the UK the number of cases diagnosed is rising. This can be accounted for by increased numbers of UK residents travelling to malaria endemic areas coupled with poor anti-malarial prophylaxis, especially by UK residents visiting their country of origin.

Brief life cycle

Sporozoites within the saliva of the vector mosquito are transmitted to the human when the mosquito feeds. These travel in the blood stream to the liver, where they infect the hepatocytes. The sporozoites develop into large 'hepatic schizonts' which contains merozoites. When the liver cell ruptures the merozoites are released into the blood where they invade red blood cells. The merozoites develop into the trophozoites, which then divide becoming a schizont containing 12-32 merozoites. The red cell ruptures releasing the merozoites into the blood stream, where they are able to invade uninfected red cells; this is associated with a temperature spike. Those merozoites which do not form trophozoites develop into male and female gametocytes, initiating the sexual stage of the life cycle. Gametocytes are found in the organs, e.g. the brain and bone marrow, and later circulate in the blood where they can be ingested by an *Anopheles* mosquito during a blood meal. The gametocytes form gametes and go through various stages in the mosquito vector before becoming sporozoites, which migrate to the mosquito salivary glands from where they can be transmitted to other humans when the mosquito takes a blood meal.

At the peak of infection, a person will carry up to two million parasites per microlitre of blood. Red blood cells infected with the trophozoite stage of *P. falciparum* sequester in capillaries of the brain, which contributes to the symptoms of cerebral malaria. In order to reduce mortality in these situations, rapid diagnosis and treatment is required.

Laboratory diagnosis of malaria

Prompt laboratory diagnosis of malaria is crucial for effective treatment. The gold standard for laboratory diagnosis is still microscopic examination of thick and thin blood films. However, in the hospital environment as well as in the field, this is time-consuming and does not allow for a quick preliminary diagnosis. The development of Rapid Diagnostic Tests (RDT), therefore, has enabled a prompt positive or negative result to be available to the clinician within minutes. The only drawback to these tests is the inability to clearly differentiate between all the human *Plasmodium* species, and such methods should always be used in conjunction with the microscopic examination of Giemsa stained slides. The primary use of such tests, therefore, is as a screening tool for obtaining a fast positive or negative result. There are a number of RDTs on the market and one of these methods is the Quantitative Buffy Coat (QBC).

Principle of the QBC method

The QBC is primarily used in African countries as it is quick, easy and gives a definitive positive or negative result. This method can also identify other parasitic infections such as Filariasis, Trypanosomiasis and Babesiosis. The QBC method does not reply on the expression of a specific surface marker or the release of an enzyme; it uses the fluorochrome Acridine Orange, which labels the DNA of the malaria parasite as well as the DNA within white blood cells. Centrifugal force is applied to the blood sample within an Acridine Orange-coated QBC capillary tube, resulting in density gradient layering of stained blood cells in conjunction with mechanical expansion of the microhaematocrit buffy coat. The infected red cells are less dense than uninfected red cells, and are thus found between the red cell and white cells layers within the spun QBC tube [Figure 1].

Figure 2. An adapted light microscope for the viewing of QBC tubes.

The QBC tube is coated with potassium oxalate, sodium heparin and K2EDTA acting as anticoagulants, and Acridine Orange, which attaches to the DNA of the malaria parasites and the nuclear material of the white cells. When excited by light of wavelength 490 nm, this exhibits a green or yellow fluorescence. The staining of the white

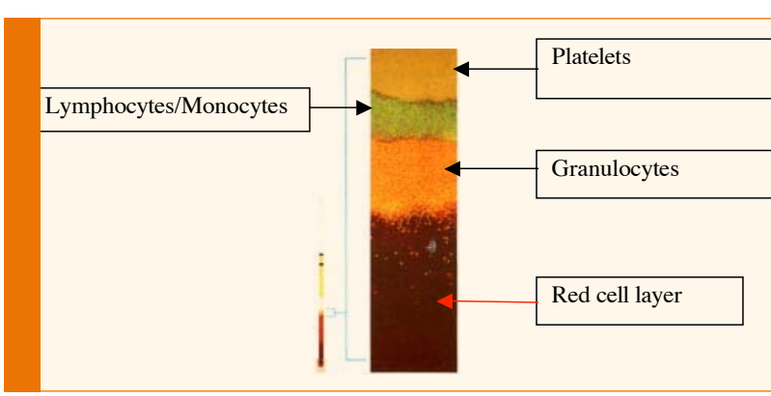

Figure 1. The viewing zone of a spun QBC tube under the microscope.

by the Acridine Orange acts as an internal control. Most of the parasites are concentrated into the narrow viewing zone of the tube. The capillary tube is inserted into a specially designed holder, namely the Paraviewer, and observed under a fluorescence microscope. An expensive fluorescence microscope is not required; an ordinary light microscope adapted by the addition of a special long-focal-length objective (paralens) connected to a light source is sufficient [Figure 2].

Method

The QBC tube is filled at the end nearest to the two blue lines; 55 to 65µL of whole blood is drawn up until the upper level is between the two blue lines [Figure 3]. The tube is then rolled between the fingers to mix the blood with the anti-coagulants. It is then gently tilted, allowing the blood to flow to the area coated in Acridine Orange. Here the QBC tube is rotated between the fingers five times to mix the blood with the stain. The tube is again tilted slightly so that the blood flows back to leave a space of approximately 6mm. With a finger over the blue line end of the tube, gentle pressure is applied to the plastic closure cap at the blood-filled end. A float is then inserted into the unsealed end, the patient's laboratory number is recorded on the sticky label provided, and placed between the two white lines on the tube. Finally the tube is centrifuged for five minutes in a parafuge to separate the different blood components, as shown in Figure 3. During centrifugation the float moves down the capillary and pushes the white cells, red cells and platelets toward the capillary surface making it easier to view under the microscope.

Trophzoites are seen as distinct bi-coloured signet rings in cells near the granulocyte layer; the central area of the signet ring is green and the ring portion is green to red in colour [Figure 4) Gametocytes of *P.falciparum* appear as yellow sickle-shaped bodies. Schizonts of *P. vivax* are recognisable by the presence of malaria pigment, which appears as a dark brown colour. Developing merozoites in mature schizonts will often be seen surrounding the dark brown pigment.

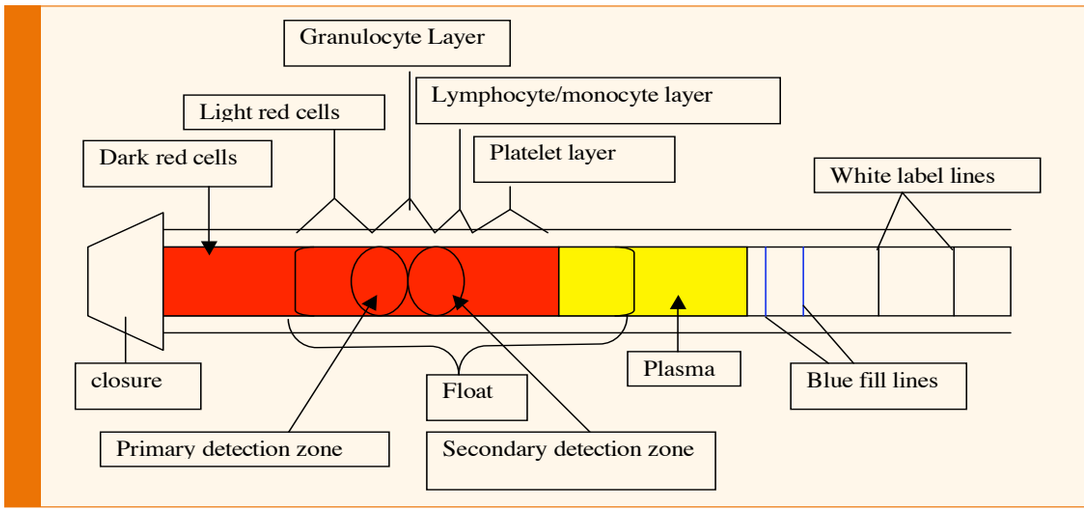

Figure 3. Schematic diagram of the QBC capillary.

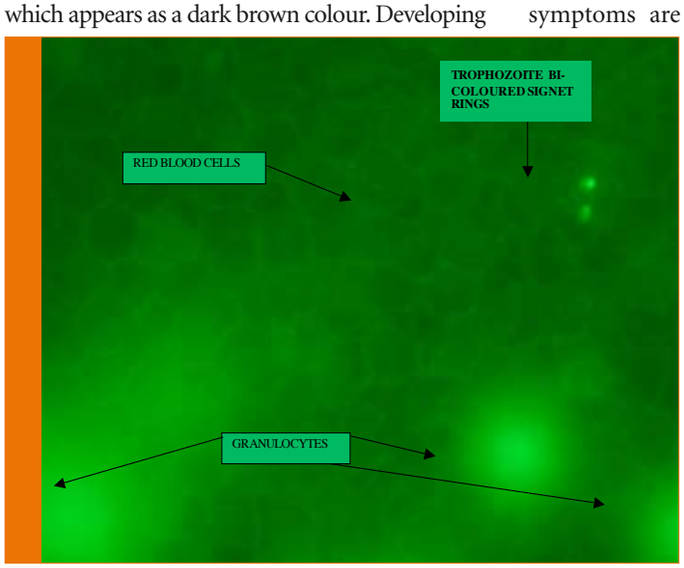

Figure 4. Trophozoites of P. falciparum.

Sensitivity and specificity

The QBC technique was compared to microscopic examination of Giemsa-stained thick blood films (GTF) under field conditions. Three hundred and sixty-four blood samples were tested, 86 were found positive and 278 negative with the GTF method, while the QBC method found 89 positive and 275 negative results.

Disadvantages/advantages of QBC

Advantages

- The method is inexpensive. Each QBC kit contains 100 tests with an average cost of £1.53 per test.
- A negative result can be established after two minutes of observation under the microscope.
- Due to the larger volume of blood observed, the method is more sensitive than the conventional GTF.
- The QBC method can be used for the diagnosis of other diseases such as Babesiosis, Trypanosomiasis and Filariasis. This is of benefit to the patient when malaria-like symptoms are caused by another parasitic infection. It enables correct diagnosis in a shorter period of time, and thus appropriate treatment.
- The QBC Test can be stored for three days after centrifugation at 16-37°C, or up to two weeks when refrigerated at 4°C prior to examination.
- Identification between a current infection and recent infection is easy to make. A recent infection will show pigment as areas of black colouration within the lymphocyte/monocyte layer only.

Disadvantages

- Species identification is not always possible and Giemsa-stained blood slides should still be examined.
- At very low levels of parasitaemia the QBC may appear negative, so blood film examination is always needed.
- Additional equipment is required e.g. micro centrifuge, adapted light microscope, paraviewer microscope holders. However, this initial cost should be recouped within 4-6 months due to low test costs.
- Red cell inclusions and large platelets will be stained with the Acridine Orange stain, but their appearance differs to that of malaria parasites.

References

1. Pinto MJW, Rodrigues SR, Desouza R, Verenkar MP. Indian Journal of Medical Microbiology 2001; 19; 219-221.
2. Bosch I, Bracho C, Perez HA. Memorias Do Instituto Oswaldo Cruz, Rio de Janerio 1996; 91; 83-86.
3. Smith AD *et al.* British Medical Journal 2008; 337; 120-126
4. Task force for of the British Committee for Standards in Haematology. Clinical Laboratory Haematology 1997;19;165-170
5. Moody A. Clinical Microbiology Reviews 2002; Jan; 66-78.

The author

Joy Gallagher MSc CSci FIBMS
Kings College Hospital
Blood Sciences Laboratory
Bessemer Wing,
Denmark Hill, London SE5 9RS, UK
Tel: +44 203 299 1687
email: joy.gallagher@kch.nhs.uk
www.kingspath.co.uk