The quantitative buffy coat for the diagnosis of trypanosomes

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SUMMARY
The use of quantitative buffy coat (QBC®) tubes developed for malaria diagnosis is described in the diagnosis of African trypanosomiasis. One hundred and thirty-four patients with *Trypanosoma gambiense* were examined using QBC® plus either haematocrit (HCT) or mini anion exchange centrifugation (MAEC) or both. QBC® was the only method that detected all 134 patients. QBC® proved to be the most sensitive diagnostic test for the detection of trypanosomes in blood. It is simple to use, gives fast results and would be a useful test at the district hospital level.

INTRODUCTION
The quantitative buffy coat technique (QBC®) was originally developed for the rapid assessment of various blood parameters using venous or finger prick blood[1]. The technique has since been extended to the diagnosis of haemoparasites[2]. QBC® has been reported as a rapid method of detecting malaria parasites[3], and more recently for the diagnosis of African trypanosomiasis[4].

The QBC® tube is a glass haematocrit tube precoated at one end with anticoagulant and at the other end with acridine orange stain (Figure 1). The tube is filled with blood, a plastic closure is attached to one end and a cylindrical float is added to the tube. The tube is centrifuged and examined microscopically using the epi-illuminated × 60 microscope objective (the ParaLens®). The components of the buffy coat separate according to their densities. As the float occupies 90% of the interior of the tube the leucocyte and platelet cell band widths and upper area of the erythrocytes are expanded to 10 times normal[1] DNA takes up the acridine orange stain and leucocyte nuclei and parasites exhibit a yellow-green fluorescence when viewed with a fluorescent microscope.

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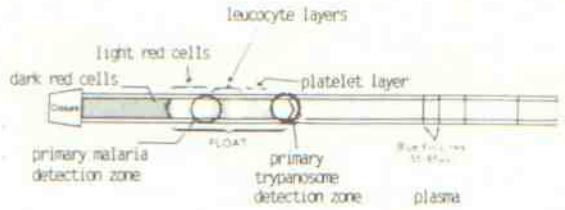

Figure 1. Diagram of a quantitative buffy coat (QBC®) tube

In early trypanosomiasis due to *Trypanosoma rhodesiense* parasites are usually easy to detect in a thick blood film, HCT is a more sensitive technique when parasites are few. In *T. gambiense*, because of the fluctuating parasitaemia and the milder clinical presentation suspects are often screened by the Card Agglutination Test for Trypanosomes (CATT). CATT positive individuals are then examined parasitologically using HCT, gland puncture and MAEC.

METHOD
A QBC® tube is filled from finger prick blood by capillary action up to the upper blue line ($\approx 65\mu l$ blood). The tube is rolled several times to ensure that the blood is well mixed with anticoagulant, and tilted to allow blood to flow to the opposite end. The tube is again rolled to mix the blood with the acridine orange dye and is sealed by placing a plastic closure on the end of the tube that contains the dye. The identification number of the sample can be written on the 'closure'. Using forceps the plastic float is inserted into the unsealed end of the tube.

Tubes are placed in the QBC® centrifuge (ParaFuge®) which automatically rotates at 12 000 rpm for 5 min. Tubes should be removed from the centrifuge and stored upright until examined. The QBC® tube is then placed in the ParaViewer® (tube holder) and 3 drops of immersion oil are placed on the tube at the plasma/buffy coat interface. The microscope stage is carefully racked up until the oil just touches the ParaLens® objective. The fine focus should be adjusted until fluorescent leucocytes are in view. It is important to ensure that the whole field is in focus. Once the tube is properly aligned it should only be moved from left to right using the microscope stage controls. The platelet/plasma interface should be examined first, followed by the platelet layer. By rotating the QBC® tube in the holder (by means of the 'closure') the whole of circumference of the

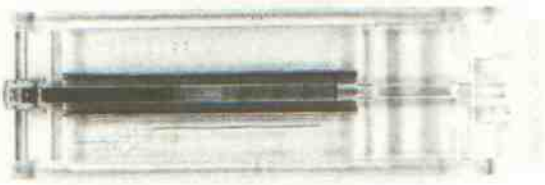

Figure 2. Qualitative buffy coat (QBC®) tube in ParaViewer holder

tube can be examined and hence it is possible to examine the entire buffy coat in a 65 μl sample of blood.

Trypanosomes are readily recognized as motile, fluorescent green organisms at the plasma/platelet interface or within the platelet layer. The movement of trypanosomes within the platelet layer is sluggish but, if the tube is left for a few minutes, most of the parasites will have moved into the plasma. The nucleus and kinetoplast fluoresce brightly. After about 20 min trypanosome motility is reduced; dead trypanosomes are red but the nucleus and kinetoplast retain their fluorescence. We recommend screening blood samples in pairs and examining both tubes before sampling further patients, to ensure that the trypanosomes are still actively motile.

The ParaLens® objective fits most microscopes, the fibre optic light module is compact and, together with a power supply or generator, enables this technique to be used in rural health centres. We would not recommend the use of the battery powered ParaFuge® centrifuge for trypanosome work. It requires recharging for 1 h after every five spins and is therefore unsuitable for field work.

Figure 3. The ParaLens epithelium illuminated objective with fibre optic light module

RESULTS

We have examined >50 patients with *T. rhodesiense* infections in Busoga, South Eastern Uganda, to assess the usefulness of the technique. In all cases trypanosomes were found very easily by QBC®. The concentration effect of the technique was impressive: often >50 parasites a field were observed with parasites frequently clumped together. Since this initial work in 1991 we tested patients with *T. gambiense* in Moyo and Adjumani, North Western Uganda, and the Côte d'Ivoire. Thirty (100%) were found positive by QBC®[4]. In a second trial in Northwestern Uganda in 1992. *T. gambiense* was confirmed parasitologically in 91 of 105 CATT whole blood positive patients. The QBC® detected all 91 and, in three individuals, it was the only positive parasitological test despite examination by HCT, MAEC and where possible gland aspiration. Similar results were found in Côte d'Ivoire in 1992. *T. gambiense* was determined parasitologically in 13 of 97 CATT whole blood positive patients. All 13 (100%) were detected by QBC® using one tube. Eleven (85%) were detected on initial MAEC and the remaining two cases were positive on a second MAEC. QBC® was used in parallel with the standard screening procedures used by the mobile teams – gland aspiration, HCT and MAEC. Overall one hundred and thirty-four *T. gambiense* patients were detected by QBC®: five of these were not detected by any other parasitological procedures.

DISCUSSION

Standard parasitological procedures used for the diagnosis of African trypanosomiasis vary according to whether the parasites are of the Gambian or Rhodesian type. CATT is useful for mass screening of large numbers of people in areas of *T. gambiense*. False positive results occur but many of these can be eliminated by testing diluted plasma rather than whole blood. Once a suspect is found CATT positive, various methods are used to identify trypanosomes. The thick blood film is normally used as a screening test in areas of *T. rhodesiense*, blood from thick film negative suspects may be further examined by HCT. The haematocrit is more sensitive than thick blood film but tubes can be difficult to examine microscopically and parasites can be missed especially if the tube is examined

too soon after centrifugation then trypanosomes may still be in the buffy layer. We would advise waiting for at least 5 min before examining HCT tubes. To date, MAEC has been considered the most sensitive concentration technique for trypanosomes[5]. The technique appears to work well in some laboratories, but in others the sensitivity is compromised by the many technical problems that can arise. Erythrocytes and fine diethyl-aminoethyl cellulose particles may pass into the collecting pipette, making detection of trypanosomes difficult. If microfilariae are present in the blood it is very difficult to examine either HCT tubes or MAEC pipettes for trypanosomes. QBC® was the only method which detected all of our confirmed trypanosomiasis cases. It is clearly the most sensitive technique. The advantages are: (1) ease of detection of parasites; and (2) standard tubes are pre-coated with anticoagulant and dye therefore technical errors are reduced. The test is simple, does not require highly trained staff to identify the motile trypanosomes and results can be obtained within 10 min of bleeding the patient. A haematocrit nomograph is available which allows a simple estimate of anaemia from the QBC® tube. Microfilariea are readily observed in QBC® tubes and are usually found within the leucocyte layers, they do not interfere with trypanosome identification. By moving the tube to the erythrocyte/leucocyte interface malaria parasites may be observed. In laboratory tests in the UK Borrelia have also been readily detected.

We conclude that QBC® is a useful technique to be employed, especially in district hospitals and rural health centres in areas of endemic sleeping sickness where passive screening of patients occurs. QBC® could replace HCT and MAEC techniques which are subject to technical problems in inexperienced hands. The role of QBC® in mass active screening programmes is less clear and requires further study.

Note

If purchased in units of 100, the QBC® tube will cost about £1.30; if purchased in units of 2000, it will be £1.11. Becton Dickinson state that the overseas price will be approximately half that of the UK purchase price.

ACKNOWLEDGMENT

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