Short Report

Direct acridine orange staining is not a 'miracle' solution to the problem of malaria diagnosis in the field

C. Delacollette[1] and **P. Van der Stuyft**[2] [1]*Projet de Lutte contre les Maladies Transmissibles et Carentielles (LMTC), Malaria Branch, Burundi;* [2]*Department of Community Health, Epidemiology Unit, Institute of Tropical Medicine, Antwerp, Belgium*

Acridine orange (AO) stains cells containing nucleic acid while leaving red blood cells unaffected. It has therefore been proposed as the basis of a rapid and sensitive method for malaria diagnosis by direct microscopical observation of blood samples (SPIELMAN *et al.*, 1988; RICKMAN *et al.*, 1989). The samples must, unfortunately, be centrifuged and strong light is needed to enhance AO fluorescence. The quantitative buffy coat system (QBC®) (LEVINE *et al.*, 1989) is, at present, the only commercial development for use 'in the field' with documented and satisfactory characteristics (COOSEMANS *et al.*, 1991; LECAMUS & RAPHENON, 1992; WONGSRICHANALAI *et al.*, 1992) The equipment necessary includes a battery operated centrifuge, capillary tubes coated with AO, a 60× oil-immersion objective that transmits ultraviolet light, or the ParaLens® system with a fibre optics cable connected to a 150 W halogen lamp (POLSUWAN *et al.*, 1992). The capillary tubes are, furthermore, expensive and the recurrent cost per test has been evaluated at 1·5 ECU (WHITE & SILAMUT, 1989; COOSEMANS *et al.*, 1991). This excludes its routine use in most developing countries. KAWAMOTO (1991) and KAWAMOTO & BILLINGSLEY (1992) have suggested an alternative, more cost-efficient AO method: direct staining of blood films with AO followed by fluorescence microscopy with a standard microscope fitted with interference filters. We have tested this approach in the *Projet de Lutte contre les Maladies Transmissibles et Carentielles* (LMTC) in Burundi.

Initially, one of us (C.D.) examined, in a rural health centre, some 70 thin blood films that had been stained with AO shortly after preparation, using a Leitz HM Lux microscope equipped with the interference filter system and the barrier filter recommended, and kindly provided, by Dr F. Kawamoto (KAWAMOTO, 1991). The slides were examined with a 40× objective and 10× eyepiece. When working with sunlight reflected by means of a supplementary flat mirror (KAWAMOTO & BILLINGSLEY, 1992) we could hardly discern malaria parasites, and the use of additional mirrors did not notably improve their visibility: the excessive ambient light hindered the observation of fluorescence and the process was further complicated by the need to adjust the mirrors in order to follow the movement of the sun and by clouds temporarily obscuring it. Adequate conditions for the examination of the blood films were eventually created indoors, by the use of an external light system: a battery-powered halogen lamp enclosed in a custom-made box (KAWAMOTO, 1991).

We then assessed, in the LMTC reference laboratory, the agreement between Giemsa staining and direct AO staining. From each of 65 blood samples we prepared 2 thin films; one was treated with Giemsa's stain for 30 min, the other with AO for 2 min. An experienced, well-trained microscopist examined the slides 'blind' and in random order. For Giemsa-stained slides we used the Leitz microscope described above with a low power lamp (10 W) and a 100× objective. For AO slides we inserted the filters and used a slide projector lamp (250 W) and a 40× objective. The thin films were examined until 1000 white blood cells (wbc) had been counted. This took an average 8 min for the Giemsa-stained slides and 4 min for AO preparations. The Table summarizes the results. The observed overall agreement was 85% (55/65). Correction for chance agreement produces a κ statistic (JENICECK & CLÉROUX, 1984) of 74%. The concordance between both procedures was perfect for parasitaemias of more than 10 asexual forms of *Plasmodium falciparum* per 1000 wbc, but it was particularly weak (3/13 or 23%) for slides with very low parasite densities, which were missed 4 and 6 times with Giemsa and AO staining, respectively. *P. malariae* parasites mixed with *P. falciparum* were detected with both methods in the same 2 blood samples.

Table. Detection of asexual forms of *Plasmodium falciparum* in 65 blood samples: comparison of results obtained using Giemsa staining and direct acridine orange staining of thin blood films[a]

		Giemsa's stain parasitaemia[b]			
	Parasitaemia[b]	0	1–10	>10	Total
Acridine orange	0	29	6	0	35
	1–10	4	3	0	7
	>10	0	0	23	23
Total		33	9	23	65

[a]Results expressed as the number of positive samples identified by the 2 techniques at the levels of parasitaemia shown.
[b]No. of parasites/1000 white blood cells.

Our results indicate that it is feasible, albeit cumbersome, to use direct AO staining for malaria diagnosis in the field. This procedure is, however, no more effective than Giemsa staining, and both approaches fall short of the superior sensitivity of the (far more expensive) QBC® method. Detection of very low parasite densities is of limited practical importance for the diagnosis of clinical malaria in the primary health care context of most developing countries. One should then consider costs in deciding between the other 2 methods. Direct AO staining is rapid and this may enable quicker and more efficient diagnostic confirmation, but in most settings time saving will not necessarily be translated into savings in labour costs. Furthermore, 175 ECU (more than the price of a good quality light microscope) have to be spent to add the light source and filters to the existing equipment or, alternatively, to obtain a fibre optic cable to direct light into the objective (MAKLER, 1991). Such an investment can be justified for laboratories at the district level or for reference centres, where direct AO staining could find a wider use. For the diagnosis of malaria in the field, however, one can happily continue to use standard light microscopes and the slower, but equally effective, Giemsa staining.

Acknowledgements

Dr F. Kawamoto personally introduced the AO technique at LMTC. His visit was financed by the World Health Organization (Tropical Disease Research)

References

Coosemans, M., Mangelschots, E. & Wéry, M. (1991). Valeur du test 'Quantitative Buffy Coat' (QBC®) comme méthode de diagnostic microscopique du paludisme. *Annales de la Société Belge de Médecine Tropicale*, **71**, 325–328.

Jeniceck, M. & Cléroux, R. (1984). *Epidémiologie : Principes, Techniques, Applications*. Paris: Edisem-Maloine, p.36.

Kawamoto, F. (1991). Rapid diagnosis of malaria by fluorescence microscopy with light microscope and interference filters. *Lancet*, **337**, 200–202.

Kawamoto, F. & Billingsley, P. F. (1992). Rapid diagnosis of malaria by fluorescence microscopy. *Parasitology Today*, **8**, 69–71.

Lecamus, J. L. & Raphenon, G. (1992). Diagnosis of malaria in the field by fluorescence microscopy using QBC® capillary

Address for correspondence: Dr P. Van der Stuyft, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerpen, Belgium.

tubes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **86**, 460.
Levine, R. A., Wardlaw, S. C. & Patton, C. L. (1989). Detection of haematoparasites using quantitative buffy coat analysis tubes. *Parasitology Today*, **5**, 132–134.
Makler, M. T. (1991). Rapid diagnosis of malaria by fluorescence microscopy. *Lancet*, **337**, 625.
Polsuwan, C., Lumlertdaecha, B., Tepsumethanon, W. & Wilde, H. (1992). Using the UV ParaLens® adaptor on a standard laboratory microscope for fluorescent rabies antibody detection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **86**, 107.
Rickman, L. S., Long, G. W., Oberst, R., Cabanban, A., Sangalang, R., Smith, J. I., Chulay, J. D. & Hoffman, S. L. (1989). Rapid diagnosis of malaria by acridine orange staining of centrifuged parasites. *Lancet*, **i**, 68–71.
Spielman, A., Perrone, J. B., Teklehaimanot, A., Balcha, F., Woodlaw, S. C. & Levine, R. A. (1990). Malaria diagnosis by direct observation of centrifuged blood samples. *American Journal of Tropical Medicine and Hygiene*, **39**, 337–382.
White, N. J. & Silamut, K. (1989). Rapid diagnosis of malaria. *Lancet*, **i**, 435.
Wongsrichanalai, C., Namsiripongpun, V., Pornsilapatip, J., Kyle, D. E. & Wilde, H. (1992). Sensitivity of QBC malaria test. *Lancet*, **340**, 792–793.

Received 22 June 1993; accepted for publication 8 September 1993

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1994) **88**, 188

Short Report

Evaluation of a malaria antigen ELISA

A. Voller[1], **D. E. Bidwell**[2] and **P. L. Chiodini**[3]
[1]*Department of Clinical Sciences, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK;* [2]*Institute of Zoology, Regent's Park, London, NW1 4RY, UK;* [3]*Hospital for Tropical Diseases, St Pancras Way, London, NW1 0PE, UK*

A test for malaria antigen was recently described by TAYLOR & VOLLER (1993) as a possible alternative or supplement to conventional Giemsa-stained blood films for the detection of malaria infections in some circumstances, and a small field study identified some potential problems with the test (NAMSIRIPONGPUN *et al.*, 1993). This report deals with a large scale evaluation of the test at the Hospital for Tropical Diseases in London.

Blood samples were obtained from patients attending the hospital for malaria investigations on the basis of symptoms or clinical history. The patients were both 'non-immune' subjects—residents of non-endemic areas who had recently returned to the UK after a short visit to the tropics, and people born in, and long-term residents of, malaria endemic areas. Because no significant difference was found between the groups in terms of malaria antigen results, they have been combined for the purposes of analysis. All patients had a blood film made and a small sample of blood was taken for routine diagnostic purposes. The blood smear was Giemsa-stained and examined by an experienced microscopist for malaria parasites. The results were provided in a coded manner for matching to the blood samples. The remainder of the blood samples, which would otherwise have been discarded, were used, without patient identification, for the serological tests. The malaria antigen enzyme-linked immunosorbent assay (ELISA) was carried out according to the method of TAYLOR & VOLLER (1993) using whole blood, containing anticoagulant, which had been frozen and thawed. The indirect malarial antibody ELISA (VOLLER *et al.*, 1974) was carried out on plasma using an extract of blood infected with *Plasmodium falciparum* as antigen.

Table. Comparison of the malaria antigen ELISA and blood slide examination

Blood films	No. examined	Malaria antigen ELISA Positive	Negative
P. falciparum	257	253	4
P. vivax	32	1	31
P. malariae	4	0	4
P. ovale	10	0	10
Negative	1400	184[a]	1216

[a]173 had evidence of previous recent experience of malaria (see text).

In total, 1703 blood samples were tested; 257 were from people with demonstrable *P. falciparum* parasitaemia (Table). Of these, 253 (98.4%) were positive in the malaria antigen ELISA. Of the remaining 4, 2 had only gametocytes in their blood films. None of the 10 patients infected with *P. ovale* or the 4 with *P. malariae*, and only 1 of 32 patients with *P. vivax* infection, gave a positive result in the antigen ELISA. The majority of the patients (1400) did not have microscopically detectable parasitaemia, but a surprising number (184) were positive in the malaria antigen ELISA. This group was investigated further and it was found that 57 of the 184 (31%) had provided a positive blood film in the previous few days, and 116 of the others (63%) gave a positive result in the malaria antibody ELISA. Thus 94% of this apparently falsely positive group had, in fact, had prior experience of malaria, confirming the finding of NAMSIRIPONGPUN *et al.* (1993) that antigen persists after parasites can no longer be found in blood films.

Overall, this study shows that the malaria antigen ELISA is highly specific and sensitive for *P. falciparum* infections, as carried out here with blood samples which have been frozen and thawed. It could, in appropriate circumstances, be used for reliable screening of large numbers of samples. The particular merit of this method is its batch processing capability, whereby hundreds of samples can be tested at the same time.

Acknowledgements
We thank the Overseas Development Administration for their support of the Appropriate Health Technology Programme at the London School of Hygiene and Tropical Medicine.

References

Namsiripongpun, V., Wilde, H., Pamsandang, P. & Tieransern, P. (1993). Field study of an antigen detection ELISA specific for *Plasmodium falciparum* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**, 32–34.
Taylor, D. W. & Voller, A. (1993). The development and validation of a simple antigen detection ELISA for *Plasmodium falciparum* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**, 29–31.
Voller, A., Bidwell, D. E., Huldt, G. & Engvall, E. (1974). A microplate method of ELISA and its application to malaria. *Bulletin of the World Health Organization*, **51**, 209–211.

Received 15 July 1993; revised 23 August 1993; accepted for publication 24 August 1993