Acridine Orange for malaria diagnosis: its diagnostic performance, its promotion and implementation in Tanzania, and the implications for malaria control

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One hundred years ago, Giemsa's stain was employed for the first time for malaria diagnosis. Giemsa staining continues to be the method of choice in most malarious countries, although, in the recent past, several alternatives have been developed that exhibit some advantages. Considerable progress has been made with fluorescent dyes, particularly with Acridine Orange (AO). The literature on the discovery, development and validation of the AO method for malaria diagnosis is reviewed here. Compared with conventional Giemsa staining, AO shows a good diagnostic performance, with sensitivities of 81.3%–100% and specificities of 86.4%–100%. However, sensitivities decrease with lower parasite densities, and species differentiation may occasionally be difficult. The most notable advantage of the AO method over Giemsa staining is its promptness; results are readily available within 3–10 min, whereas Giemsa staining may take 45 min or even longer. This is an important advantage for the organization of health services and the provision of effective treatment of malaria cases.

The national malaria control programme of Tanzania, together with the Japan International Co-operation Agency, began to introduce the AO method in Tanzania in 1994. So far, AO staining has been introduced in 70 regional and district hospitals, and 400 laboratory technicians have been trained to use the method. The results of this introduction, which are reviewed here and have several important implications, indicate that AO is a viable alternative technique for the laboratory diagnosis of malaria in highly endemic countries.

Malaria continues to be one of the world's most significant health problems, accounting for 300 million–500 million clinical attacks and over 1 million deaths every year — mainly in children under 5 years of age (WHO, 1999). Although malaria remains a considerable public-health threat in parts of Asia and South America, 90% of its current burden is concentrated in sub-Saharan Africa. Here, malaria is responsible for 20%–30% of hospital admissions and 30%–50% of outpatient consultations (http://mosquito.who.int/docs/prescriber18_p1.pdf). In areas where transmission is intense, malaria creates an array of biological and behavioural responses with long-term impacts on social and economic growth and development (Sachs and Malaney, 2002). The growing recognition of the extent and impact of malaria on socio–economic development has prompted calls for greater investment, with an overall goal of improving health, particularly among the poor (Jha *et al.*, 2002). The main aim of the recently announced 'Global Fund to Fight HIV/AIDS, Tuberculosis and Malaria' is to support technically sound and cost-effective interventions for the prevention, treatment

and care of the worst affected communities. These interventions are laudable and include increased access to health services, provision of critical health products, including drugs, training of personnel and community health workers, and the promotion of behavioural change, outreach, and community-based programmes (http://www.globalfundatm.org/overview.html).

The resurgence of malaria, commencing in the 1970s after the abandonment of the global eradication campaign that mainly relied on chemical vector control, was accompanied by a more clinical approach towards malaria control. The revised, global, malaria-control strategy put forth in the early 1990s stressed the accessibility of case management, the provision of early diagnosis, and prompt treatment. This strategy, which was described as a basic right of populations wherever malaria occurs (WHO, 1993), has been accepted and implemented by all malaria-endemic countries and has already led to the retraining of $>16,000$ health providers (WHO, 2000*b*). Effective case management depends on both accurate and rapid diagnosis. Given the low specificity of diagnostic algorithms based only on clinical signs and symptoms, there is a great need for widely available and simple methods of parasite detection. The method that is currently the most widely used is the microscopical examination of Giemsa-stained bloodsmears. Although this method was developed about 100 years ago, it has low direct costs (once the microscopes are purchased), shows high sensitivity and specificity, and can be used to estimate levels of parasitaemia, to identify the *Plasmodium* species present, and to reveal non-malarial blood parasites. However, Giemsa staining employs multiple reagents, requires well trained and experienced personnel and is labour intensive, somewhat messy and time-consuming (typically taking at least 45 min from blood collection to the result). Use of the method may lead to under-estimates of parasite densities (if parasites are lost as the smears are washed). Giemsa staining is not capable of automation and might induce fatigue, which in turn can lead to false diagnoses (WHO, 2000*a*).

Many alternative approaches to Giemsa staining have been developed over the past 20 years, as molecular methods, including techniques for the detection of malarial antigen or pigment, have evolved (Hänscheid, 1999). However, none of these methods has been able to replace Giemsa staining as the most routinely used technique, mainly because of their high costs, which put them beyond the reach of a large proportion of patients. Furthermore, some of the techniques are difficult to implement or exhibit only a moderate sensitivity. Several methods have been described that use a fluorescent dye for microscopical malaria diagnosis. So far, the most extensively studied dye is Acridine Orange (AO). The AO method is relatively straightforward, and most importantly, the diagnostic results are available within a few minutes. The simple design of an interference filter, to be mounted on light microscopes, has made direct AO staining a simple and economically viable method for malaria diagnosis (Kawamoto, 1991*b*).

Below, the literature that led to the discovery, development and use of AO fluorescence microscopy is summarized. The diagnostic performance of AO is compared with that of more conventional staining methods, in a variety of epidemiological settings. The results of attempts to promote, implement and sustain the AO method in Tanzania are discussed. Finally, the potential implications of introducing the AO method into primary-healthcare facilities in developing countries, for the diagnosis and control of malaria, are outlined.

DEVELOPMENT OF AO FLUORESCENCE MICROSCOPY

The first microscopical diagnosis of malaria using thin bloodsmears probably occurred in 1880, with the discovery of parasites in the erythrocytes of a malaria patient. Further

progress was achieved with the development of a dehaemoglobinized, thick-smear preparation in 1902, which facilitated the examination of 20 times more blood than used in a thin smear and increased the sensitivity of parasite detection (Ross, 1903). Several stains were employed for the detection of malarial parasites, such as Romanowsky's (in 1891), Leishman's stain (in 1901) and Giemsa's (in 1902). The latter proved to be the best all-round stain for malaria diagnosis (Payne, 1988). Today, exactly 100 years after its initial development, the Giemsa staining of thick and thin smears is still the most commonly applied technique for the routine diagnosis of malaria in virtually all places where the disease is endemic. The thin smear is used to quantify and identify the parasite species involved, whereas analysis of the relatively large volume of the blood used to make the thick smear enhances the sensitivity of the diagnosis.

Though fluorescent dyes were widely applied in botanical studies in the beginning of the last century, their first use for the identification of human parasites was only reported in the 1930s: first for trypanosomes (in 1935) and then for malarial parasites (in 1939). Bock and Oesterlin (1938–1939) studied the use of several dyes for the visualization of different protozoa, including *Plasmodium knowlesi*, *P. ovale* and *P. vivax*, and reported excellent results using a 0.1% solution of Rivanol (an acridine derivative). The effectiveness of differentiating parasites with Rivanol was confirmed for *Haemoproteus* when Patton and Metcalf (1943) tested six different dyes. The basic feature of fluorescent staining is that, in contrast to the mature erythrocytes that do not contain DNA or RNA, the nucleic acids of the parasite fluoresce strongly. Intra-erythrocytic malarial parasites are rich in RNA, which fluoresce red after staining with AO, whereas other fixed elements in the smear appear green or unstained. If the pH is below 6.4, the parasites will stain orange, whereas at higher pH fluorescence is less intense and unspecific (Richards *et al.*,

1969). The microscopist, however, must learn to distinguish the stained cells of the parasite from other stained cells containing nucleic acids, such as leucocytes or erythrocytes containing Howell Jolly bodies (which might be seen in patients with anaemia; Moody, 2002). After testing 30 different fluorescent dyes, Fuhrmann (1962) found an AO solution with a pH between 6 and 7 and a concentration of 1: 10,000 to be the most promising dye for the staining of thin bloodsmears containing *Babesia canis*, *P. berghei* or *P. cathemerium*. The use of AO on thick films, however, was reported to be difficult, because of the intense staining of the leucocytes (Fuhrmann, 1962). However, shortly thereafter, excellent results were reported for the AO staining not only of thin smears containing *P. berghei* or *P. vivax* (Ambroise-Thomas *et al.*, 1965) but also of thick smears containing various *Plasmodium* species (Sodeman, 1970). In the latter study, the thick smear was made slightly thinner than was then customary and dehaemoglobinized during staining with a 0.01% AO solution of pH 5.4 (Sodeman, 1970). Commonly, AO stain is applied to a thin smear only after the smear has been dried and then fixed in methanol. However, an immediate method, in which AO solution is applied directly to an unfixed and undried blood film, has also been described (Kawamoto, 1991a; Metzger and Nkeyi, 1995).

The first application of AO for the identification of malarial parasites outside the laboratory was described in 1973 (Shute and Sodeman, 1973). In this study, AO and Giemsa staining were compared, using thick smears obtained from hospital-based and epidemiological surveys (see Table). Shute and Sodeman (1973) concluded that AO did not present sufficient advantages over Giemsa to justify its more general use. Perhaps because of this conclusion or the relatively high costs of fluorescence microscopes at the time, no further studies on the use of fluorescent dyes in the diagnosis of malaria were conducted for another 18

years. In 1991, however, the development of a relatively cheap, interference-filter system (a multi-layered excitation filter combined with a barrier filter) designed for use with AO in a standard light microscope provided a low-cost tool for malaria diagnosis employing AO (Kawamoto, 1991*b*).

A combination of centrifugation, AO staining and fluorescence microscopy was developed into the quantitative buffy coat (QBC) method. Although this technique appears to be easy to handle, sensitive and rapid, it requires costly pre-prepared tubes, a centrifuge and an ultra-violet microscope (Lema *et al.*, 1999). It is not being discussed here in more detail.

DIAGNOSTIC PERFORMANCE OF AO

The published literature contains the results of several valid attempts to evaluate the diagnostic performance of the AO staining of bloodsmears for malaria diagnosis (see Table). Most of these studies were carried out in high- and low-prevalence settings of Africa, India, the Philippines and Thailand, but one trial analysed bloodsmears from travellers returning to France who were suspected to have malaria (Gay *et al.*, 1996). The results of a study from Indonesia are excluded from the Table because the authors (Syafruddin *et al.*, 1992) acknowledged difficulties with the quality of diagnosis in the field. In all settings, the AO method was found to be easy to perform, although Craig and Sharp (1997) reported some problems in staining their smears, and Lema *et al.* (1999) found the light of the microscope's lamp to be uncomfortable to their eyes.

The AO method showed a good diagnostic performance when compared with Giemsa's or other staining solutions, with sensitivities of 81.3%–100%, specificities of 86.4%–100% and positive and negative predictive values of 76.0%–100% and 87.8%–100%, respectively. The diagnostic performance of the AO method became worse as the size of the parasites present

(Shute and Sodeman, 1973) or the intensities of the parasitaemias (Shute and Sodeman, 1973; Gay *et al.*, 1996; Craig and Sharp, 1997) decreased. However, when Gay *et al.* (1996) compared the sensitivities of AO and Giemsa staining for detecting light parasitaemias (<1000 parasites/µl), they found that AO was significantly more sensitive than Giemsa (50.0% *v.* 14.3%; with QBC as the 'gold standard').

With AO staining, identification of the *Plasmodium* species present in a smear may occasionally be difficult because vacuoles, pigments and chromatin dots are often not apparent (Midha *et al.*, 1981). In addition, the precise morphology of the red blood cells is often not clearly visible (Gay *et al.*, 1996). Kawamoto (1991*a*) successfully identified *P. malariae* and *P. ovale* in AO-stained smears, but this seems exceptional. In *P. vivax* infections, AO stains the RNA of the invaded reticulocyte as well as the RNA of the parasite, which facilitates detection of the parasites (Makler and Gibbins, 1991). Gay *et al.* (1996) reported that 85.2% of the *Plasmodium* species present in their smears were correctly identified after AO staining (when compared with the results of Giemsa staining). A similar level of agreement (86%) was reported in the detection in smears of *Plasmodium* species other than *P. falciparum*, when AO was compared with Giemsa by Lowe *et al.* (1996). All comparative appraisals emphasised the rapidity of the AO method. From the time of blood collection, AO staining can give a result within 3–10 min, whereas Giemsa staining usually takes 45 min or even longer (Tarimo *et al.*, 1998).

PROMOTION AND IMPLEMENTATION OF THE AO METHOD FOR MALARIA DIAGNOSIS IN TANZANIA

In Tanzania, although few other countries in sub-Saharan Africa have such a large number of health posts staffed by trained

personnel, malaria remains one of the leading causes of morbidity and mortality (Font *et al.*, 2001) and clinical malaria diagnosis, without microscopical confirmation, is commonly practised, even in regional and district hospitals. In a holo-endemic area of malaria transmission such as Tanzania, an inability to differentiate malaria episodes from other febrile illnesses results in considerable over-diagnosis of malaria (Rooth and Björkman, 1992). One reason why there is such a reliance on the clinical diagnosis of malaria in Tanzania is the inability of any diagnostic laboratory in the country to check, using the relatively time-consuming Giemsa-staining procedure, more than 40–50 suspected malaria cases/day (Anon., 2000).

In the 1990s, as the AO method had been found to have good diagnostic performance and to be much more rapid than Giemsa staining, the national malaria control programme of Tanzania, in collaboration with the Japan International Co-operation Agency (JICA), launched a project to promote the AO method throughout Tanzania. Between 1994 and 1999, 70 hospitals were equipped with modified fluorescence microscopes (normal light microscopes, each supplied with halogen lamp and a pair of filters) to perform the AO method. The objective was to standardize AO staining for the microscopical diagnosis of malaria in selected regional and district hospitals of Tanzania. Two-week training courses were held to introduce laboratory technicians to the new diagnostic method. Each course consisted of: an 'overview' lecture (on the biology of the malarial parasites and malaria diagnosis); instruction on the preparation of thin and thick bloodsmears; review of staining methods based on Giemsa's or Field's stain; instruction on AO staining and on the handling and management of microscopes fitted with halogen bulbs; and a review of methods of quality assurance in diagnosis and reporting. Eight courses have been conducted to date, and some 400 technicians have been trained.

Simple questionnaires were sent to all 70 participating hospitals in 1999, to evaluate the utilization of the AO method. Almost all (95%) of the hospitals responded that they would wish to employ the AO method as their preferred technique for day-to-day malaria diagnosis, and 40% of the hospitals had already fully adopted AO staining as their routine practice. In full agreement with previous results, the AO method was found to be quick and accurate. The mean time to perform malaria diagnosis with AO was 3 min, as opposed to at least 60 min with the formerly used Giemsa staining. This rapidity is an important factor, as the demand for microscopical confirmation of suspected malaria in Tanzanian hospitals is very high. The questionnaire-based survey revealed that 27.1% of the hospitals had never used the AO method. In addition, about 25% of the hospitals that had used the AO method upon its introduction were no longer using it because of missing accessories and/or unavailability of supplies (e.g. AO powder for the solution, light bulbs, slides and cover glasses, spare parts to repair broken microscopes). Some technicians also felt that they had insufficient knowledge to perform the AO method. The lack of a stable electricity supply was also frequently reported. About half of the hospitals that reported that they were using the AO method also reported that they were only using their 'AO' microscope one to 50 times/month; this relatively low frequency was explained by a lack of supplies or a lack of stable electricity. In hospitals with adequate supplies and more stable electricity, AO microscopes were being utilized for >8 h/day.

DISCUSSION

A diagnostic test for malaria that is useful in the humid tropics must not only have good sensitivity and specificity but also needs to be affordable, rapid and easy to use. It should also give a measure of the level of parasitaemia, as asymptomatic malarial

TABLE. *The performance of Acridine-Orange (AO) staining in the diagnosis of malaria caused by* Plasmodium falciparum, P. vivax *or other* Plasmodium *species*

Study and setting	Sample size and (preparation)	Malaria prevalence (%)	'Gold standard'	Diagnostic performance of AO v. 'gold standard' (%)				Parasitaemia	Species identification	Reference
				Sensitivity	Specificity	PPV	NPV			
San Lazaro hospital, Manila; field surveys, Philippines	339 patients (thick smears)	47.1	Giemsa	100 (*P. vivax*); ND (*P. falciparum*)	100 (*P. vivax*); ND (*P. falciparum*)	100 (*P. vivax*); ND (*P. falciparum*)	100 (*P. vivax*); ND (*P. falciparum*)	Accuracy declined as trophozoite counts fell; difficulties in detecting young parasites	Satisfactory	Shute and Sodeman (1973)
Rohtak, India	500 patients (thin and thick smears)	43.0	Romanowsky	94.3	94.5	92.6	95.8	Possible difficulties in detecting small parasites or low parasitaemias using AO	Satisfactory	Midha *et al.* (1981)
Two rural malaria clinics, Thailand	236 patients (thin and thick smears)	41.1	Giemsa	ND	ND	ND	ND	Not known	Not known	Wongsrichanalai *et al.* (1992)
Three clinical laboratories, Khartoum, Sudan	77 patients (thin and thick smears)	27.2	Giemsa	ND (AO: 21 positive; Giemsa: five positive)	ND	ND	ND	Not known	Not known	Agabani *et al.* (1994)
Rural health centre, Burundi	65 patients (thin smears)	49.2	Giemsa	81.3	87.9	86.7	82.9	Low accuracy of both methods at low parasite densities	Predominance of *P. falciparum*	Delacollette and van der Stuyft (1994)
Kilifi district hospital, Kenya	200 patients (thin and thick smears)	70.5	Giemsa	97.7	86.4	94.5	94.4	For parasitaemias of <100 parasites/µl, AO had a sensitivity of 83.3% and a specificity of 86.5%	Other than *P. falciparum*: AO detected 86% correctly	Lowe *et al.* (1996)
Hospitalier Pitié-Salpêtrière, Paris, France	243 travellers returning to France (thin smears)	50.6	Giemsa	87.0	95.8	95.5	87.8	Direct correlation between sensitivity and parasitaemia found; at low parasitaemia, AO had higher sensitivity than Giemsa	AO not satisfactory for species identification (morphology of erythrocytes not clearly visible)	Gay *et al.* (1996)
Manguzi Hospital, Kwa Zulu Natal, South Africa	125 patients; 26 treated previously (thin and thick smears)	55.5	Giemsa	93.1 (thick film); 82.8 (thin film)	97	98.1	93.5	No significant difference between four techniques in dilution study with varying parasite densities	Predominance of *P. falciparum*	Craig and Sharp (1997)

Study and setting	Sample size and (preparation)	Malaria prevalence (%)	'Gold standard'	Diagnostic performance of AO *v.* 'gold standard' (%)				Parasitaemia	Species identification	Reference
				Sensitivity	Specificity	PPV	NPV			
Three district hospitals, Dar es Salaam, Tanzania	400 patients (thin and thick smears)	52.3	Giemsa	94.3	100	100	94.1	With parasitaemias of <5000 parasites/µl, AO had a sensitivity of 90.2%	Predominance of *P. falciparum*	Tarimo *et al.* (1998)
Two health centres, one district hospital, Machakos and Makueni district, Tanzania	213 patients (thin and thick smears)	24.4	Field and Giemsa	86.5*	89.5*	76.0*	94.5*	Sensitivity decreased to 55% with low parasitaemia (fewer than four rings/100 leucocytes)	Predominance of *P. falciparum* (exception: one *P. ovale* case)	Lema *et al.* (1999)
India	2420 patients (thick smears)	10.2	Leishman	59.7 (Leishman *v.* AO)	ND	ND	ND	Not known	Not known	Hemvani *et al.* (1999)

*Mid-points of ranges given by authors in original publication.
PPV, Positive predictive value; NPV, negative predictive value; ND, not determined by original authors and cannot be calculated from published data.

infections are pervasive in highly endemic areas. Diagnosing malaria by conventional light microscopy — usually by examination of Giemsa-stained bloodsmears — is currently the 'gold standard' in most parts of sub-Saharan Africa. As this method itself reveals limitations of sensitivity, particularly at low parasite densities, the assessment of the diagnostic performance of any new method against Giemsa staining is problematic. For example, examination of 200 bloodsmears taken from patients in Kenya revealed 141 positive by Giemsa but 146 positive by AO (Lowe *et al.*, 1996). It may be difficult to determine whether cases who are positive by AO but negative by Giemsa are true- or false-positives. Such questionable cases should perhaps be evaluated by a third method, or perhaps the definition of the 'gold standard' needs to be modified. A very large discrepancy was observed between the results based on AO staining and those based on Giemsa staining when the blood samples being checked came from Europeans who had returned from trips to Africa and typically carried < 5000 malarial parasites/µl blood; of 69 samples confirmed positive by QBC, 43 were positive by AO but only 21 were found positive after Giemsa staining (Gay *et al.*, 1996).

Whenever the diagnostic performance of AO has been evaluated for different levels of parasitaemia, a decrease in sensitivity has always been observed at lower parasite densities (Shute and Sodeman, 1973; Gay *et al.*, 1996; Craig and Sharp, 1997). This clearly compromises the usefulness of the AO method. In areas where malaria is holoendemic, however, it may be sufficient to detect all of those with relatively high parasitaemias, since they are the only individuals likely to develop any clinical symptoms of malaria (Smith *et al.*, 1994). In such settings, a failure to detect some of the low parasitaemias may have no public-health significance. On the other hand, misdiagnoses are of considerable concern in hypo-endemic areas. Here, conventional Giemsa staining, characterized by a detection threshold of just five

to 10 parasites/µl blood, is still recommended as the method of choice.

There are several rapid diagnostic tests commercially available that are based on the detection of malarial antigen. These may be an appropriate alternative to Giemsa and AO staining, especially as they require no extra equipment and only minimal training. However, there is currently concern about inter-batch variation in these test kits, with some batches apparently showing insufficient specificity and sensitivity (Wongsrichanalai and Miller, 2002). In the study of 169 pre- and post-treatment samples by Rubio *et al.* (2001), the OptiMal (Flow Incorporated), ParaSight-*F* (Becton Dickinson) and ICT Pf/Pv (AMRAD ICT) kits all displayed high frequencies of false-positives and false-negatives and all lacked sensitivity at low levels of parasitaemia. Similarly unsatisfactory results were obtained in Myanmar with the OptiMal and ICT Pf/Pv kits (Mason *et al.*, 2002). The antigen-detection kits are also more expensive than smear staining. Treatment-seeking behaviour is already largely influenced by costs, with smaller percentages of patients among poor communities presenting for diagnosis and treatment than among better-off communities (Biritwum *et al.*, 2000). Malaria diagnosis should be affordable to all patients.

The most notable advantage of AO over Giemsa staining is its rapidity. This has at least three important consequences for malaria diagnosis and control. Firstly, the AO method might be an appropriate diagnostic tool for health centres in developing countries that are visited by many patients with suspected malaria or by many drug-resistant cases, as well as for large-scale screening in epidemiological surveys. Secondly, prompt diagnosis facilitates the provision of better and more client-friendly services to the patients, as waiting times are significantly reduced. Currently, bloodsmears are often not handled immediately but rather processed in batches, so that many patients can only be told whether they are smear-positive hours after they have given a blood sample

or on the following day (WHO, 2000*a*). The demand on the laboratory technician's time may be such that microscopical confirmation of malaria has to be restricted to severely affected patients (WHO, 2000*a*). Consequently, many patients are prescribed antimalarials before or without any laboratory confirmation, to provide symptomatic relief. Malaria diagnosis based only on clinical signs and symptoms is notoriously difficult, as the fever, nausea, headache and/or chills that occur in malaria are not malaria-specific and overlap with several other febrile illnesses. After Chandramohan *et al.* (2002) conducted a review of the literature on the presumptive diagnosis of malaria in different epidemiological settings, they considered that between 39% and 96% of the clinical diagnoses of malaria were erroneous. Thus, the majority of the subjects were treated with antimalarials unnecessarily. Although Chandramohan *et al.* (2002) developed algorithms with improved diagnostic performance, by combining predictors or employing a scoring system, their algorithms were still insufficiently sensitive and specific.

The third and probably most important consequence of the rapidity of the AO method for malaria diagnosis and control is that rapid (and accurate) diagnoses could cut the unnecessary use of antimalarial drugs and lead to better treatment of those who do have symptomatic malaria. At present, self-treatment is widespread in sub-Saharan Africa and the general failure of those who treat themselves to adhere to adequate treatment regimens is well acknowledged (McCombie, 1996). It is not just the quality of health care and service available that contribute to an individual's treatment-seeking behaviour (in terms of, for example, which public-health facility to attend for diagnosis, or whether to delay treatment or simply purchase the drugs he or she considers appropriate) but it is also the time diagnosis and treatment may take. The longer diagnosis and treatment take, the more time the ill individual loses. The time that could other-wise have been spent in productive activity or relaxation, together with economic factors such as transportation costs, all influence treatment choice. Self-treatment minimizes expenditure and time lost, especially if diagnosis is so slow that the ill individual would otherwise have to wait for hours at the health facility or present again. Unfortunately, drugs purchased outside the official health sector (e.g. in local shops or kiosks) are often of low quality, sub-standard or even counterfeit. In a recent study in Thailand, 40% of the drug samples analysed fell outside pharmacopeial limits (Shakoor *et al.*, 1997). Slow diagnosis thus encourages self-treatment (usually with inadequate doses of low-quality drugs) and the consequences of this are many-fold. In general, drug resistance develops most rapidly when sub-therapeutic doses of antimalarial drugs filter out the resistant forms of the parasite (White, 1998*a*). Inadequate doses, inappropriate dose regimens or poor compliance, especially of drugs with long terminal elimination phases, invariably lead to rapid selection of resistant parasites. Multi-drug resistance is already a major threat to effective malaria control in many parts of South–east Asia. The high levels of chloroquine resistance found over large parts of sub-Saharan Africa have prompted several countries to switch to sulfadoxine–pyrimethamine (SP) as the first-line drug, but levels of SP resistance are also rapidly increasing. Cross-resistance between compound classes further amplifies the problem. With the single exception of artemisinin derivatives, resistance has emerged to all other antimalarial drugs readily available on the market (White and Olliaro, 1996). Hence, the rational use of antimalarial drugs, either in monotherapy or, ideally, as combined treatments, is mandatory to prolong the time that the drugs will remain effective (White *et al.*, 1999). Over-prescribing, self-treatment and the treatment of asymptomatic cases should all be reduced.

Presumptive malaria diagnoses often result in the treatment with antimalarial

drugs of non-malarial cases. Unnecessary adverse effects and high costs accompany the over-administration of antimalarials. Presumptive treatment was less problematic before resistance to chloroquine became widespread, because this drug was cheap, safe and non-toxic. Prescriptions of quinine and/or SP may cause unnecessary adverse effects among non-infected patients. Quinine is extremely bitter and may induce nausea, tinnitus or high-tone deafness. Compliance is poor under unsupervised conditions (White, 1998b). In addition, these drugs are more costly than chloroquine. In a Malawian hospital, Jonkman *et al.* (1995) demonstrated that U.S.$14,000 could be saved annually if only those confirmed to be malaria cases by microscopy (rather than all suspected cases of malaria) were treated with SP.

In conclusion, the promotion and implementation of the AO method in 70 regional and district hospitals of Tanzania is under way, with the majority of the hospitals favouring this new diagnostic approach and 40% already fully adhering to the AO method. However, several problems were encountered, such as a lack of supplies, unstable electricity or a high frequency of broken bulbs on delivery. There is clearly a need for constant surveillance and monitoring and careful organization of the shipment of supplies and the local procurement of materials. The use of daylight-illuminated or battery-powered microscopes fitted with interference filters could avoid the problems associated with the halogen bulbs and unstable supplies of electricity. After the logistic problems have been resolved, AO might evolve as the method of choice for malaria diagnosis, not only in Tanzania's hospitals but also in many other parts of sub-Saharan Africa.

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