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Diagnosis of malaria using thick bloodsmears: definition and evaluation of a faster protocol with improved readability

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The value of some inexpensive modifications to the standard method of preparing thick bloodsmears, involving rapid drying, an isotonic fixative and a haemolysing solution containing saponin, was evaluated. The drying, haemolysing, fixing and staining steps, together called the fast-thick-smear method (FTS), can be completed in <10 min. The FTS and a more classical thick-smear method (CTS) were both used on each of 1185 samples of venous blood samples from 1034 cases of suspected malaria (all international travellers returning to France). The results indicated that there was no statistically significant differences between the two methods in terms of their sensitivity, specificity or predictive values for parasite detection. However, estimates of the intensities of the *Plasmodium falciparum* infections observed, based on counts of trophozoites against 200 leucocytes, were markedly higher (37.8% higher overall) with the FTS than with the CTS ($P < 0.0001$). Moreover, the concordance between results obtained by inexperienced and experienced microscopists was excellent when the FTS was used, with a kappa value of 0.96 (95% confidence interval = 0.93–0.98).

Malaria is no longer rare in temperate, non-endemic areas. For example, despite the known gross under-reporting of malaria, Muentener *et al.* (1999) identified 77,683 cases of imported malaria in Europe and 15,010 more in North America, all of which were reported between 1985 and 1995. *Plasmodium falciparum* was the most frequently identified cause, accounting for 82.2%, 56.2% and 38.5% of the known cases in France, the U.K. and the U.S.A., respectively (Muentener *et al.*, 1999). Given the potential severity of the clinical pathology induced by this species — 1.62% of the known imported cases of *P. falciparum* malaria in Europe as a whole, 0.49% of those in France, and 1.01% of those in the U.S.A. were fatal — diagnosis of the infection needs to be rapid and reliable and any clinical laboratory should be able to perform this diagnosis at any time of the day (Danis *et al.*, 1999; Muentener *et al.*, 1999). Ideally, a clinician should have the results of the examination of a blood sample for malarial parasites within 2 h of the sample's collection (Anon., 1999). The World Health Organization's 'gold standard', and the accepted laboratory procedure for the diagnosis of malaria, is the preparation and examination under the light microscope of blood (collected from a finger-prick or by venipuncture) that has been prepared as thin and thick smears and stained with Giemsa's or Field's stain (Payne, 1988; Warhurst and Williams, 1996). The classical method of preparing thin bloodsmears is fast and easy to perform but infections in which there are fewer than

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150 trophozoites/μl are rarely detected in thin smears and microscopical examination of such smears can be very time-consuming when parasitaemias are low. The old thick-smear technique, described by Ronald Ross almost a century ago (Ross, 1903), is 10–30 times more sensitive, with a detection threshold of about five to 15 trophozoites/μl (Trape, 1985; Moody and Chiodini, 2000). The main advantage of the thick smear in malarial diagnosis is that it allows a greater volume of blood to be visualized, per unit area of smear per unit time, than the thin smear. Theoretically, it would require, on average, 30 times longer to find a parasite in a thin smear than in a thick smear of blood from the same patient. As many cases of imported malaria present with low parasitaemia (10%–20% having <100 parasites/μl), even microscopists in clinical laboratories in non-endemic areas should be able to prepare and interpret both types of smear (Anon., 1996; Van den Ende, 1998; Mills *et al.*, 1999). However, there are two main problems with thick smears. Firstly, the detection threshold is highly dependent on the observer's experience, because of the distorted appearance of the parasites and the background interference left when the erythrocytes are lysed in water; this is a particular problem in areas where malaria is rare. Secondly, the preparation of traditional thick smears is time-consuming (often up to 12 h) because of the time taken in drying and haemolysing the sample. Despite these two problems, the 'Ross' thick-smear technique has undergone very little improvement in the past 100 years. Although many variations in the drying, haemolysing, staining or examination steps have been proposed, no modification of the old protocol has been widely adopted. The fact that study centres now often use slightly different modifications of the old technique may make a valid comparison of their results difficult.

For several years, staff at the Centre Hospitalier–Universitaire Pitié-Salpêtrière (CHUPS) in Paris have been trying to improve the appearance, and reduce the preparation time, of thick bloodmears. Three main modifications of the technique are apparently of potential interest in reaching this goal. The first two — preparing each smear with just 2-μl of blood and drying it in an incubator at 37°C — became routine at the CHUPS several years ago and permitted drying times to be cut to just a few minutes. The detection threshold of this technique, hereafter called the classical thick-smear method (CTS), was determined to be just five trophozoites/μl blood (Gay *et al.*, 1994). More recently, a third modification was introduced: the use of saponin to lyse the erythrocytes in the thick smear. This modification not only leads to a very short preparation time but also improves the appearance of the parasites and decreases the level of background interference. The addition of saponin lysis to the CTS has produced a method known as the fast-thick-smear method (FTS). The main aim of the present study was to evaluate the FTS, in comparison with the CTS.

MATERIALS AND METHODS

The CTS and Development of the FTS

The FTS was based on the CTS, a method that has been used at the CHUPS, unchanged, for the last 7 years. In the CTS, 2 μl of well homogenized, venous, whole blood are transferred onto a clean microscope slide and spread out to give a thick, circular smear measuring 5–7 mm in diameter. The slide is placed in an incubator at 37°C until the smear is dry (for 10–15 min), then covered with water for 7–15 min (to allow complete haemolysis), rinsed in water, immediately stained, for <30 s, using a commercial kit based on eosin and methylene blue (RAL 555; Réactifs RAL, Paris, France), and finally dried, using an electric fan, for 5–10 min. The total time needed to prepare a CTS smear for microscopical examination is <40 min.

In the development of the FTS from the CTS, the optimal conditions for drying

the smear at 37°C and lysing and staining the smear were ascertained. The effect of lysis using water was compared with that of lysis using the saponin solution recently described by Petithory *et al.* (1997). The optimal composition of the lysis solution was determined by varying the concentration of each component: saponin (Sigma–Aldrich) was used, at a concentration of 1, 2, 3, 4 or 5 g/litre, in physiological saline (pH 6.8) containing 1.5%, 3% or 6% of a commercial fixative (pH 4.0) consisting of 35% formaldehyde stabilized with 10% methanol (Soyons Chimie Biotechnologie, Soyons, France). The use of Triton X-100, Tween 20 or Thimerosal (all Sigma–Aldrich) instead of the saponin was also investigated, each at the concentrations proposed by Petithory *et al.* (1997) for the saponin. For each lysis solution tested, the time necessary to obtain complete red-cell lysis was measured. Two Giemsa-based staining procedures — staining with the commercial kit used in the CTS or a fast-acting Giemsa solution (Réactifs RAL) — were also compared, with or without a preliminary fixative step using different concentrations (10%, 20%, 30%, 40%, 50%, 75% and 100%) of methanol in water.

Once the optimum lysing solution had been determined, the cost of 5 ml of it (i.e. the additional cost/smear of replacing the CTS with the FTS) was estimated.

Prospective Study

STUDY SITE

The study was conducted in the Parasitology–Mycology unit of CHUPS, where >1500 blood samples are checked for malarial parasites each year (between June 1999 and June 2000, for example, 1888 blood samples were investigated and 20% were found positive for *Plasmodium*).

SUBJECTS AND SAMPLES

The 1034 subjects were international travellers who had developed malaria-like symptoms shortly after visiting malaria-endemic areas and had then been referred to CHUPS in January–February 1999, June 1999 or September 1999–February 2000. A 5-ml sample of venous blood was collected from each patient, into a tube with EDTA, and immediately transported to the Parasitology–Mycology unit for analysis.

METHODOLOGY

Optimization of the FTS led to a technique that, with the CTS and the routine preparation of thin smears [which were fixed in methanol and stained with the fast-acting Giemsa solution (diluted 1:16 in tap water; pH 7.35) for 2 min], was applied to each sample (see Results).

The 21 microscopists who took part in the study were categorized as expert (three individuals with >10 years' experience of malaria diagnosis), experienced (four individuals with >2 but <8 years' experience) or inexperienced [(14 individuals either with no experience (11) or 6 months' experience (three)]. Before checking any samples, the inexperienced microscopists received 6 h of training in the FTS technique.

Each blood sample was examined on-site within 1 h of its collection. For both the FTS and CTS, thick smears were examined under Laborlux binocular microscopes (Leitz, Wetzlar, Germany) with built-in illumination and at a total magnification of ×1000 (×10 wide-field eyepieces and a ×100 oil-immersion lens). A thick smear was only considered negative after the entire surface of the smear had been checked and no parasites had been found. Thin smears, also checked at a total magnification of ×1000, were only deemed negative if no parasites had been seen after 20 min of checking (equivalent to about 700 fields). The study was blinded: all the CTS and thin smears were read by one experienced microscopist, all the FTS slides were read independently by another experienced microscopist, and most (632) of the FTS smears were re-read, again independently, by an inexperienced microscopist. The results were collected

each day. Whenever the results from the two examiners checking the same FTS smear or the results for the CTS and FTS smears of the same sample were discordant, the relevant smears were each re-examined by two experts, using a blinded protocol. If a thick smear initially reported as negative was then deemed positive, the presence of parasites on it was confirmed by a third expert. The final results, known as the results corrected by experts (RCE), were used as the gold standard.

The level of parasitaemia in any case who was found positive for *P. falciparum* but not with an intense infection was estimated using the FTS smear (once by an experienced microscopist and sometimes again, independently, by an inexperienced microscopist) and once with the CTS (by the experienced microscopist who had checked the corresponding FTS smear). An infection was considered 'intense' if at least one parasite was detected, at a total magnification of $\times 1000$, in four random fields on the corresponding thin bloodsmear; this roughly corresponds to a parasitaemia of $\geqslant 0.1\%$, or $\geqslant 4000$ parasites/μl. In general, levels of parasitaemia were estimated from the thick smears by counting the malarial parasites against 200 leucocytes and multiplying each count to give the number of parasites/μl blood (assuming each patient had 8000 leucocytes/μl blood; Warhurst and Williams, 1996). When the count against 200 leucocytes was zero (i.e. the parasitaemia was <40/μl), all the parasites in the 2-μl smear were counted and the count simply halved to give the number of parasites/μl.

The quality of the CTS smear and the corresponding FTS smear, of each of 30 consecutive malaria-positive samples, was evaluated independently by two experienced microscopists (one, chosen at random for each sample, checking the FTS smear and the other the CTS). The examiners were asked to score the adherence of the smear, the completeness of lysis, the preservation of the parasites and the level of background interference as 1 (bad), 2 (acceptable) or 3 (good).

The time taken to deliver a result to the attending clinician, measured from the time the blood sample was drawn, was measured for the CTS and FTS smears produced from 100 consecutive blood samples and checked by experienced microscopists.

DATA ANALYSIS

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the thin-smear, CTS and FTS methods, in the detection of infection with *Plasmodium* sp., were estimated for all the subjects, using the RCE results as the gold standard. The kappa statistic (κ; Fleiss, 1981) was used to measure the levels of agreement between the experienced and inexperienced microscopists who examined the same FTS smears and those between the RCE results and those collected, by experienced microscopists, using thin, CTS or FTS smears.

The paired estimates of parasitaemia were compared using the Wilcoxon matched-pairs test, and differences giving *P*-values of <0.05 were considered statistically significant.

All the data analyses were performed using version 5.0 of the Stat-View software package (SAS Institute Inc., Cary, NC).

RESULTS

Determination of the Optimal Conditions for the FTS

The optimal time to dry the 2-μl thick smear in the 37°C incubator was found to be 2–3 min. The best results for lysis were obtained with 0.4%–0.5% (w/v) saponin solution. Although the addition of formaldehyde/methanol to the lysis solution did not change the quality of the preparation, in terms of the completeness of lysis, the state of preservation of parasites or the amount of background interference, it did improve the adherence of the smear to the slide and also prevented bacterial contamination of the solution when it was stored for more than a few days; at 1.5% or 3% the formalin/

methanol gave the same results, which were better than those seen with 6%. Although use of the RAL 555 kit and fast-acting Giemsa solution produced equally well stained smears, the RAL 555 kit was considered more useful as it could be used to stain smears more rapidly (<30 s *v.* >120 s).

These results led to the development of the optimal lysis solution and the finalized FTS method used in the prospective study. The lysis solution (pH 6.2) was prepared by mixing 400 ml of physiological saline (pH 6.8) with 5 g saponin and 15 ml 35% formaldehyde/10% methanol (pH 4.0) and then making the volume up to 1000 ml with more saline; this solution was stored at 4°C until needed. The procedure for the FTS was broken down into four simple steps: smear preparation (as for the CTS); drying (2–3 min at 37°C incubator); haemolysis (immersion in the optimal lysis solution for 2–4 min, followed by a rinse in water); and staining using the RAL 555 kit (three, 1-s dips in the eosin, a rinse in water, four, 1-s dips in the methylene blue, then a rinse in water before the smear was dried using an electric fan).

The cost of the optimal lysing solution was U.S.$0.125/test (i.e. U.S.$2.5/litre).

Prospective Study

Overall, 1185 whole-blood samples were collected from the 1034 subjects. The RCE indicated that 269 (22.7%) of the 1185 samples were positive for *Plasmodium* sp., the infections being identified, by morphology, as purely *P. falciparum* (84.0% of the positive samples), *P. ovale* (8.2%), *P. malariae* (3.0%) or *P. vivax* (2.6%), a mixture of *P. falciparum* and *P. ovale* (1.1%), or of a *Plasmodium* sp. that could not be identified (1.1%). Seventy-three (27.1%), three (1.1%) and one (0.4%) of the 269 RCE-positive samples had been considered negative by the experienced microscopists who examined thin, CTS and FTS smears of the samples, respectively. All of these false-negatives results were associated with low parasitaemias. The sensitivities, specificities, predictive values and κ-values for the thin, CTS and FTS smears are summarized in Table 1.

The use of CTS and FTS to estimate the numbers of *P. falciparum* trophozoites/μl blood was compared using 103 blood samples (Table 2). In addition, the use of both thick-smear methods to estimate the densities of *P. falciparum* gametocytes was compared using another 24 samples (Table 2). The estimated mean trophozoite density was 37.8% higher when based on FTS smears checked by experienced microscopists than when it was based on CTS smears checked by microscopists with similar experience of malaria diagnosis ($P < 0.0001$). Although the corresponding difference in the estimates of gametocyte density was less (10.5% higher for FTS), this was still statistically significant ($P = 0.0022$). However, trophozoite densities estimated on 64 FTS smears by experienced microscopists were almost identical to those estimated by inexperienced microscopists using the same smears ($P = 0.2227$).

The mean overall scores for the quality of preparation were 8.70 for each CTS smear and 11.17 for each of the FTS. The mean values for each of the four criteria of quality (CTS *v.* FTS) were 2.07 *v.* 2.73 for good adherence, 2.73 *v.* 2.83 for completeness of lysis, 2.00 *v.* 2.80 for state of preservation of the parasites, and 1.90 *v.* 2.80 for the amount of background interference.

The median times (and ranges) necessary to perform the techniques and for the results to reach the attending clinician were 55 (35–180) min for the CTS and 45 (20–180) min for the FTS, with corresponding arithmetic means of 66 and 54 min, respectively.

According to the RCE, 160 (25.3%) of the 632 samples that were each checked twice as FTS smears (once by an experienced microscopist and once by an inexperienced) were positive for malarial parasites: 133 (83.1% of the positives), 14 (8.7%), five (3.1%) and four (2.5%) with pure *P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax* infections, respectively, two (1.3%) with mixed *P. falciparum*/*P. ovale* infections

TABLE 1. *The results of experienced and inexperienced microscopists examining thin bloodsmears, 'classical' thick smears (CTS) and 'fast' thick smears (FTS) for* Plasmodium infections, *using the results corrected by experts (RCE) as the gold standard*

		No. of positives		No. of negatives		Sensitivity	Specificity	Predictive value (%)		κ-value and (95% confidence interval)
Smear	Microscopist	True	False	True	False	(%)	(%)	Positive	Negative	
COMPARISON OF METHODS ($N = 1185$)										
Thin	Experienced	196	0	916	73	72.9	100	100	92.6	0.805 (0.763–0.848)
CTS	Experienced	266	0	916	3	98.9	100	100	100	0.990 (0.981–1.000)
FTS	Experienced	268	0	916	1	99.6	100	100	100	0.997 (0.993–1.000)
COMPARISON OF MICROSCOPISTS ($N = 632$)										
FTS	Inexperienced	159	9	463	1	99.4	98.1	94.6	99.8	0.959 (0.932–0.977)
FTS	Experienced	160	0	472	0	100	100	100	100	1.000

TABLE 2. *Estimates of the level of asexual-stage (trophozoite) and sexual-stage (gametocyte)* Plasmodium falciparum *parasitaemia, based on 'classical' thick smears read by experienced microscopists (CTS) and 'fast' thick smears read by experienced microscopists (FTSexp) or inexperienced microscopists (FTSinexp)*

Parasite stage	*N*	Arithmetic mean of estimated parasitaemias (parasites/μl)			Difference in estimates (%)	*P*
		FTSexp	FTSinexp	CTS		
Trophozoites	103	1155	–	730	37.8	<0.0001
Trophozoites	64	1114	1139	–	2.2	0.2227
Gametocytes	24	180	–	161	10.5	0.0022

and two (1.3%) with a *Plasmodium* sp. that could not be identified. The results obtained by the experienced microscopists using FTS agreed perfectly with the gold standard, with no false-negative or false-positive results apparent. The results for the inexperienced microscopists, who only scored one (0.6%) of the 160 RCE-positive samples as negative and nine (1.9%) of the 472 RCE-negative samples as positive, were almost as good. The one false-negative result was associated with low parasitaemia (two trophozoites/μl). The inexperienced microscopists made a few mistakes in identifying the *Plasmodium* sp. involved in the infections they detected. Two (1.5%) of the 135 cases infected with *P. falciparum* (either alone or with *P. ovale*) were not correctly identified — one case, with mixed *P. falciparum*/*P. ovale* infection, was reported as a pure *P. ovale* infection, and a *P. falciparum* infection with old trophozoites containing pigment (>150/μl) was mis-identified as *P. malariae* — and the *Plasmodium* sp. involved in each of three (17%) of the 18 samples from patients with pure *P. ovale* or *P. vivax* infections was also mis-identified.

DISCUSSION

It would be useful to improve the low-cost, basic and reliable techniques that are available for malaria diagnosis. High-technology approaches are often too expensive for use in many situations, especially in developing countries. For a small additional cost, the optimized FTS described gives a better preparation than the CTS while remaining simple and rapid. Although each of the main steps of the FTS — the use of saponin as lysing agent, the rapid drying of the smear and the use of a known volume of blood to produce the smear — has been investigated in the past, an integrated analysis of these parameters has not been performed before.

Although recommended by Bruce-Chwatt (1984), the small sample of blood used to produce each FTS smear, 2 μl, may seem small compared with the 5–20 μl normally recommended for thick smears. However, part of the FTS protocol is to examine the entire 2-μl smear before deeming it negative, whereas, in the standard method of examining a thick smear, it is usual to examine no more than 100–200 microscope fields (at ×1000), which correspond to only 0.25–0.5 μl of blood (Dowling and Shute, 1966; Trape, 1985; Chiodini, 1998). The parasite detection threshold for the CTS, which is also based on a 2-μl smear, is about five parasites/μl (Gay *et al.*, 1994) and apparently represents the highest level of sensitivity ever reported for the microscopical examination of thick bloodsmears (Bruce-Chwatt, 1984).

Use of an incubator at 37°C cuts the time needed to dry an FTS smear to just 2–3 min. Although shorter drying times (1–2 min) can be achieved using a microwave oven (Chevallier *et al.*, 1992), this appears to have a detrimental effect on the smear's readability, leading to relatively low estimates of the level of parasitaemia (Le Goff *et al.*, 1998).

Inclusion of saponin in the lysis solution is supported by its long use, in fundamental

research, to liberate intra-erythrocytic malarial parasites from their host cells (Christophers and Fulton, 1939; Kreier, 1977). Saponin makes cells permeable by forming complexes with membrane cholesterol (Bangham and Horne, 1962). It is more than six decades since Christophers and Fulton (1939) reported that malarial parasites appeared similar whether erythrocytic lysis had been achieved using water or saponin, and that parasites from saponized fresh blood where infective to mice. Surprisingly, the potential use of saponin in malaria diagnosis was not evaluated until the 1970s and even then the sample — five patients infected with *P. vivax* — was very small (Ulmas and Fallon, 1971). After another 26 years, Gleeson (1997) and Petithory *et al.* (1997) outlined the usefulness of cytoconcentration in the detection of blood parasites and described suitable techniques in which haemolysis with saponin was followed by centrifugation. Gleeson (1997) centrifuged samples in a standard laboratory centrifuge, limited the evaluation to eight cases of malaria, and failed to report the stages or species of *Plasmodium* involved. Petithory *et al.* (1997), using a cytocentrifuge and evaluating their technique on 57 malaria-positive samples (46 of which were positive for *P. falciparum*), achieved $>100\%$ sensitivity (by detecting *P. falciparum* in three samples considered negative by the gold standard) and correctly identified the *Plasmodium* sp. involved in each case. However, they did not conduct a prospective evaluation and reported a major problem with their technique: infections in which only young trophozoites of *P. falciparum* were present in the peripheral blood could be missed because such small trophozoites did not centrifuge down very well. Since these two cytocentration-based techniques were described, only the Gleeson method appears to have been used in an epidemiological study (John *et al.*, 1998). It seems likely that the use of a centrifugation step, particularly one using a cytocentifuge (a rare commodity in most malaria-endemic areas), is a complication that limits the appeal of either technique as a method for the routine diagnosis of malaria.

The present results failed to demonstrate that the sensitivity of the FTS (in terms of the detection of infection) was significantly better than that of the CTS. However, there are several indications that FTS is a useful improvement on the CTS. FTS, for example, revealed three cases that were misdiagnosed using the CTS, and experienced microscopists were more likely to give concordant results when they used FTS than when they used CTS. Inexperienced microscopists using the FTS produced results (for both the prevalence of infection and the level of parasitaemia) that were very close to those of experienced examiners checking smears produced with the same technique. Moreover, the estimates of the level of *P. falciparum* parasitaemia based on examination of FTS smears were significantly higher than those produced using CTS smears, particularly when the counts were of trophozoites (Table 2). The gametocytes (measuring approximately 5–12 μm) are much larger than the early trophozoites (1–2 μm in diameter) and consequently more easy to pick out and to identify on a microscopic field, even when distorted by water haemolysis. It is probably the young trophozoites that are easier to see on FTS smears than on CTS smears; the three cases misdiagnosed using CTS all had parasitaemias composed solely of young trophozoites whereas the only case misdiagnosed using FTS had a low parasitaemia (one parasite/μl) composed solely of gametocytes.

The results for the inexperienced microscopists show some interesting features. Their only false-negative result was associated with a very low parasitaemia (two trophozoites/μl). All of their nine false-positive results were recorded in the first month of the study and were caused by artefacts or Howell–Jolly bodies (data not shown). When considering the excellent results achieved by the 'inexperienced' examiners, two facts need to be remembered: (1) not all of these

examiners were totally without experience (three had each spent 6 months in another parasitology unit and had checked 25–132 bloodsmears for malaria before the present study); and (2) the smears they checked were not examined in the stressful context of emergency care, where more mistakes, particularly false-positive results, occur. In general, the inexperienced microscopists were not able to discriminate reliably between the four *Plasmodium* spp. infecting humans, even when they used FTS smears. The sensitivity for *P. falciparum*, for example, was <98% (133/135); two individuals were incorrectly recorded as not being infected with *P. falciparum*, even though one of them had >150 old trophozoites of *P. falciparum*/μl. This could be problematic in terms of patients leaving the emergency unit with unsuitable chemotherapy.

In general, the present results should encourage the use of the FTS method, although several questions need to be answered before the widespread and routine use of this new technique is recommended. The method's usefulness (alone or as a confirmation of the result of a dipstick assay) in the routine diagnosis of malaria by inexperienced or experienced microscopists — who are under pressure to get a result to the clinician in attendance — remains to be evaluated.

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REFERENCES

Anon. (1996). A rapid dipstick antigen capture assay for the diagnosis of falciparum malaria. *Bulletin of the World Health Organization*, **74,** 47–54.

Anon. (1999). Dealing with and preventing imported malaria due to *Plasmodium falciparum*. Short text of recommendations. *Médecine et Maladies Infectieuses*, **29** (Suppl. 2), 109–114.

Bangham, A. D. & Horne, R. W. (1962). Action of saponin on biological cell membranes. *Nature*, **196,** 952–953.

Bruce-Chwatt, L. J. (1984). DNA probes for malaria diagnosis. *Lancet*, **i,** 795.

Chevallier, B., Cavallo, J. D., Baudet, J. M., Samson, T., Gros, P., Crenn, Y. & Meyran, M. (1992). Diagnostic rapide du paludisme. Le four à micro-ondes. *Bulletin de la Société de Pathologie Exotique*, **85,** 223–225.

Chiodini, P. L. (1998). Non-microscopic methods for diagnosis of malaria. *Lancet*, **351,** 80–81.

Christophers, S. R. & Fulton, J. D. (1939). Experiments with isolated malaria parasites (*Plasmodium knowlesi*) free from red cells. *Annals of Tropical Medicine and Parasitology*, **33,** 161–170.

Danis, M., Legros, F. Gay, F., Brousse, G., Bricaire, F. & Gentilini, M. (1999). Paludisme d'importation en France. *Médecine et Maladies Infectieuses*, **29** (Suppl. 3), 257–273.

Dowling, M. A. C. & Shute, G. T. (1966). A comparative study of thick and thin blood films in the diagnosis of scanty malaria parasitemia. *Bulletin of the World Health Organization*, **34,** 249–267.

Fleiss, J. L. (1981). *Statistical Methods for Rates and Proportions*, 2nd Edn. New York: John Wiley & Sons.

Gay, F., Traore, B., Zanoni, J., Danis, M. & Gentilini, M. (1994). Evaluation du système QBC pour le diagnostic du paludisme. *Santé*, **4,** 289–297.

Gleeson, R. M. (1997). An improved method for thick film preparation using saponin as a lysing agent. *Clinical and Laboratory Haematology*, **19,** 249–251.

John, S. M., Sudarsanam, A., Sitaram, U. & Moody, A. H. (1998). Evaluation of OptiMal^{TM}, a dipstick test for the diagnosis of malaria. *Annals of Tropical Medicine and Parasitology*, **92,** 621–622.

Kreier, J. P. (1977). The isolation and fractionation of malaria-infected cells. *Bulletin of the World Health Organization*, **55,** 317–331.

Le Goff, G., le Hesran, J. Y. & Robert, V. (1998). Le four à micro-ondes pour le séchage des gouttes épaisses. Intérêt et limites dans l'observation microscopique des trophozoïtes et des gamétocytes de *Plasmodium falciparum*. *Bulletin de la Société de Pathologie Exotique*, **91,** 214–216.

Mills, C. D., Burgess, D. C., Taylor, H. J. & Kain, K. C. (1999). Evaluation of a rapid and inexpensive dipstick assay for the diagnosis of *Plasmodium falciparum* malaria. *Bulletin of the World Health Organization*, **77,** 553–539.

Moody, A. H. & Chiodini, P. L. (2000). Methods for the detection of blood parasites. *Clinical and Laboratory Haematology*, **22,** 189–202.

Muentener, P., Schlagenhauf, P. & Steffen, R. (1999). Imported malaria (1985–95): trends and perspectives. *Bulletin of the World Health Organization*, **77,** 560–566.

Payne, D. (1988). Use and limitations of light microscopy for diagnosing malaria at the primary health care level. *Bulletin of the World Health Organization*, **66,** 621–626.

Petithory, J. C., Ardoin, F., Ash, L. R., Vandemeulebroucke, E., Galeazzi, G., Dufour, M. & Paugam, A. (1997). Microscopic diagnosis of blood parasites following a cytoconcentration technique. *American Journal of Tropical Medicine and Hygiene*, **57,** 637–642.

Ross, R. (1903). An improved method for microscopical diagnosis of intermittent fever. *Lancet*, **i,** 86–87.

Trape, J. F. (1985). Rapid evaluation of malaria parasite density and standardization of thick smear examination for epidemiological investigations. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **79,** 181–184.

Umlas, J. & Fallon, J. N. (1971). New thick-film technique for malaria diagnosis. Use of saponin stromatolytic solution for lysis. *American Journal of Tropical Medicine and Hygiene*, **20,** 527–529.

Van den Ende, J., Vervoort, T., Van Gompel, A. & Lynen, L. (1998). Evaluation of two tests based on the detection of histidine rich protein 2 for the diagnosis of imported *Plasmodium falciparum* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **92,** 285–288.

Warhurst, D. C. & Williams, J. E. (1996). Laboratory diagnosis of malaria. *Journal of Clinical Pathology*, **49,** 533–538.