Diagnosis of malaria in a remote area of the Philippines: comparison of techniques and their acceptance by health workers and the community

David Bell,[1] Rouel Go,[2] Cynthia Miguel,[2] John Walker,[3] Lillibeth Cacal,[4] & Allan Saul[1]

Objective To compare the efficacies of remote symptom-based diagnosis of malaria, rapid diagnostic tests and microscopy in an area of low endemicity in the Philippines.
Methods In Trial I, 350 symptomatic patients were tested within their villages using malaria *Plasmodium falciparum* (Pf)/*Plasmodium vivax* (Pv) immunochromatographic tests (ICT tests) and blood films stored and read under local conditions. The slides were later restained and read. In Trial II, unsupervised volunteer barangay health workers prepared ICT tests and slides after brief training. These slides were read at rural health units. Twenty-seven barangay health workers and 72 community members were later questioned about the three diagnostic strategies.
Findings A history of fever alone was sensitive (95.4%) but poorly specific (16.5%) for predicting parasitaemia. The inclusion of other symptoms reduced the sensitivity to below 85%, while specificity remained low. The axillary temperature was poorly predictive. ICT tests achieved high sensitivity (97.9%) but many cases indicated as positive by ICT tests were negative by microscopy. Further analysis of these cases in Trial I indicated that ICT tests were detecting low-level parasitaemias missed by microscopy, and that local microscopy had poor accuracy. ICT tests were well accepted and accurately performed by barangay health workers.
Conclusion These tests meet a strong desire in the community for blood-based diagnosis and may increase the compliance and treatment-seeking behaviour of patients.

Keywords Malaria/diagnosis; Diagnostic techniques and procedures; Blood chemical analysis; Chromatography; Microscopy; Signs and symptoms; Voluntary workers; Comparative study; Clinical trials; Philippines (*source: MeSH*).

Mots clés Paludisme/diagnostic; Techniques et procédés diagnostiques; Analyse chimique sang; Chromatographie; Microscopie; Signes et symptômes; Travailleur bénévole; Etude comparative; Essai clinique; Philippines (*source: INSERM*).

Palabras clave Paludismo/diagnóstico; Técnicas y procedimientos diagnósticos; Análisis químico de la sangre; Cromatografía; Microscopia; Signos y síntomas; Trabajadores voluntarios; Estudio comparativo; Ensayos clínicos; Filipinas (*fuente: BIREME*).

Bulletin of the World Health Organization, 2001, **79**: 933–941.

Voir page 940 le résumé en français. En la página 940 figura un resumen en español.

Introduction

The increasing global toll of malaria has spurred new interest in the development of community-based strategies against the disease (*1–3*). Despite evidence of the cost-effectiveness of improving treatment access and compliance (*4*), most victims of malaria still die because of a lack of health care close to their homes or because their condition is not diagnosed by health workers (*5*, *6*). The delivery of treatment in remote areas is complicated by the centralized nature of microscopy services. Diagnosis based on symptoms alone has inherent difficulties (*6*, *7*), although volunteer health workers in remote areas have practised it with some success (*3*, *8–10*). Over the past few years, developments in rapid field diagnostic techniques based on the demonstration of parasite antigens have opened new possibilities for improved remote malaria diagnosis that is independent of centralized diagnostic services (*11–18*).

A reliable test for use in remote malaria diagnosis should be highly sensitive, affordable, stable and easy to use with minimal support and training, and it should have a high negative predictive value so as to minimize overtreatment. A number of products now available demonstrate high sensitivity

[1] Australian Centre for International and Tropical Health and Nutrition, The Queensland Institute of Medical Research; and The University of Queensland, Royal Brisbane Hospital Post Office, Qld Australia 4029 (email: davidbe@qimr.edu.au). Correspondence should be addressed to David Bell at the latter address.

[2] Research Institute for Tropical Medicine, Alabang, Muntinlupa City, Philippines.

[3] Department of Parasitology, Westmead Hospital, Westmead, Sydney, Australia.

[4] Rural Health Unit, Esperanza, Agusan del Sur, Philippines.

Ref. No. **00-0802**

and specificity for the rapid detection of circulating *Plasmodium falciparum* (Pf)-specific histidine-rich protein 2 (HRP-2) (*16–19*). Products detecting *Plasmodium vivax* (Pv) parasitaemia are now available or under development. The malaria Pf/Pv immunochromatographic test (ICT test) (Amrad-ICT, Sydney, Australia) is similar to the ICT Malaria Pf test (*13*, *18*) with the additional detection of a pan-specific malaria antigen (P-S Ag) common to *P. falciparum* and *P. vivax*. In the ICT text, 15 µl of finger-prick blood are flushed along a glass-fibre strip by a reagent provided with the kit. Parasitaemia is determined by the appearance of a control band and one or both antigen bands (P-S Ag and HRP-2 Ag) within 10 minutes.

Most studies of rapid diagnostic tests, including those of the ICT test, have involved comparisons with centralized microscopic diagnosis performed in clinics and laboratories (*11*, *20*). The present study assesses the reliability and acceptability of the ICT test in a remote area of the Philippines, where volunteer health workers with limited training and support diagnose and treat malaria, and compares it with local microscopic diagnosis and symptom-based diagnosis.

Material and methods

Study site

The study was performed in Agusan del Sur Province in the north-east of the island of Mindanao. Malaria occurs principally in the more remote areas and is characterized by generally low perennial transmission of *P. falciparum* and *P. vivax*, with pockets of higher transmission. The economy is based on semisubsistence agriculture, the population living in scattered small farms and villages in barangays (administrative districts) of 2000 to 3000 people. Microscopy services are provided in the rural health units (RHUs) in the larger towns, and travel to these is difficult and expensive. All barangays have volunteer barangay health workers (BHWs) who have received basic training in common diseases, including malaria. They work from health centres or from home and are supervised by paid midwives.

This study took place within the Agusan del Sur Malaria Control and Prevention Project (ADS-MCP), which aims to develop a community-based, self-sustainable malaria control programme covering approximately 450 000 people at risk. As part of the programme, BHWs receive additional training to administer chloroquine, free of charge, to symptomatic patients who fit a standardized diagnostic algorithm that includes fever and headache and/or chills or rigors occurring within the preceding three days. At the time of treatment the BHWs prepare blood films. These are sent to the RHUs and the results are delivered to the BHWs for statistical and educational purposes. Delays of several weeks frequently occur between the preparation of slides and the time when they are stained and read, and this leads to autofixation and opacification of the thick films because of the warm humid conditions (*21*).

Trials

Two trials comparing ICT tests with locally prepared slides were conducted in five barangays during 1998 and 1999. In the first trial an assessment of symptoms was also performed. Patients were recruited in accordance with the ADS-MCP algorithm, and also included were people with a more distant history of fever or nonspecific symptoms or signs suggestive of malaria (e.g. headache only). Chills and rigors are considered to be synonymous with the term *hilanat* in the predominant local language, Visaya, and are included under the term "chills" below. As few prevalence data were available, sample size was determined by the number of eligible cases attending in the time available.

Trial I was performed from September to November 1998. Two visits lasting two to four days were made to each barangay. Patients were questioned regarding the nature and duration of recent symptoms. ICT tests and slides were prepared and the ICT tests were read by the researchers. The intensity of ICT antigen bands was graded 1 (faint) to 3 (equal to or darker than the control band). The slides were read by an experienced local microscopist, who was not aware of the results of the ICT tests, in accordance with WHO criteria (100 thick film fields before negativity declared). This was done after storage and transportation for between two and four weeks at ambient temperature and humidity, as was usual in the area. Positive results included asexual or sexual parasite stages; in *P. falciparum* both of these stages express HRP-2 (M. Parra, personal communication). Staining was performed with fixative and Quick-dip stain (FAME Analytical Reagents and Laboratories, Manila, Philippines).

Slides of all discordant cases, i.e. where the ICT results differed from the slide results, all cases ICT-positive for *P. vivax*, and 20% of cases negative by slide and ICT, were washed, soaked in buffer and restained in dilute Giemsa by a procedure adapted from techniques described previously (*21*, *22*) to reverse the effects of autofixation. These slides were read again by an experienced parasitologist at Westmead Hospital, Sydney.

Trial II began in September 1998 and continued for 12 months. The technique for the ICT test was demonstrated, written instructions in Visaya were issued, and supervision of the BHWs was provided when they were working with their first one or two patients. Local BHWs carried out the recruiting of patients, the ICT tests and the preparation of slides. Diagnoses were later checked and results with no clear control band were excluded. The slides were stained and read at the RHUs and the results were subsequently retrieved. Only 113 of 368 microscopy results could be matched with ICT results by name and date. Patients treated during the four weeks preceding the tests were excluded from analysis.

The ICT tests used in Trial I were refrigerated until two weeks before use, while those in Trial II were stored by BHWs at room temperature, averaging about 25 °C, for up to six months. They were all from the same batch and were used before the expiry date indicated by the manufacturer.

Axillary temperatures were recorded for 248 of the 351 patients in Trial I, and for 109 of the 368 patients seen by BHWs in Trial II, using standard digital thermometers (Becton Dickinson, USA).

Acceptability survey

Six months after the introduction of ICT tests in Trial II, 27 of the 35 BHWs involved were individually asked the following questions. *What do you think about the use of slides (ICT tests)? Do slide results (ICT test) alter your work? If so, how?*

A total of 71 adults from four villages, two of them with resident BHWs, were systematically selected on the basis of village size, and were questioned on their preferences for investigation and treatment for fever in a hypothetical adult. For this purpose, three picture cards showing the following choices were presented in random order: blood slide and treatment according to symptoms; rapid diagnostic test at a cost of US$ 0.60 and treatment according to result; treatment according to symptoms, with no pathological investigation.

Data analysis and consent

The study was explained and consent was obtained in Visaya from all participants. All interviews were conducted in Visaya. Ethical approval was obtained from the Research Institute for Tropical Medicine, Manila, Philippines, and the Queensland Institute for Medical Research, Brisbane, Australia.

The results were double-entered and analysed by means of EpiInfo 6.04c (*23*). Measures of diagnostic accuracy of the ICT tests and microscopy were calculated in relation to each other as concordance, i.e. % identical results. Parasitaemia demonstrated by either microscopy or ICT test was then used as a standard to compare microscopy, ICT test and symptom-based diagnosis. This involved the assumption of a low false-positive rate and therefore of a high overall specificity of the ICT test and microscopy.

Results

Demographic and illness data

The sex ratios of the populations differed between the two trials (Table 1). More males were seen by the BHWs in Trial II ($\chi^2 = 8.49$, $P = 0.004$). The mean ages were similar (F-statistic <0.01, $P = 0.95$).

Of the 334 patients in Trial I with no history of treatment within the previous four weeks, 302 (90.4%) reported fever within the previous three days, while 253 (75.7%) reported chills and 270 (80.8%) reported headache. Of the 32 patients with no recent fever, 31 had a recent history of headache and/or chills, and one had pallor.

Practical aspects of use of ICT test

Few problems were experienced in the preparation of the ICT test. The most common one was flooding with reagent, resulting in poor blood flow up the glass-fibre strip. When this happened the tests were repeated. There was a rapid fall in the frequency of these occurrences as experience was gained. Twenty-five of 393 tests collected from the BHWs were considered invalid because of indistinct control bands; 22 were done in the first two months of the trial (invalid rate 8%), and three were done later (invalid rate 1.8%). Most invalid tests probably resulted from flooding. The control bands in Trial I were all well defined.

Seven tests in Trial I developed broad coloured bands because the flow of reagent deposited chromatic material from the control band below the HRP-2 and P-S Ag bands. Although distinct from truly positive results, they could be mistaken as positive by inexperienced readers.

A qualitative reduction in control band intensity, and that of the HRP-2 and P-S Ag bands, was noted late in Trial II. Variation in the storage and delivery of tests prevented the assessment of changes in sensitivity with time.

Comparisons of ICT and slide results

ICT results prepared and read by the researchers in Trial I and by the BHWs in Trial II are shown with the corresponding microscopy results in Table 2, including Trial I microscopy results after restaining. Although not prepared in ideal conditions, restained slides were considered adequate by an expert microscopist.

Table 1. **Demographic details of patients and parasitaemia as indicated by ICT test**

Trial	Sex (*n*)			Mean age (years)					Mean temperature (°C)				
	n	M	F	*n*	Total	ICT+	ICT-	*P*-value	*n*	Total	ICT+	ICT-	*P*-value
I	350	171 (58.5)[a]	179 (56.4)	350	19.5	16.4	23.7	<0.001	247	37.2	37.4	37.0	<0.001
II	113	73 (61.6)	40 (67.5)	113	19.8	17.7	23.4	0.08	22	38.8	39.0	38.5	0.27

[a] Figures in parentheses are the percentages of patients with parasitaemia.

Table 2. **Matched results of 350 ICT tests and slides in Trial I, and 113 ICT tests and slides in Trial II**

ICT result	Trial I (first reading)					Trial I (re-read)						Trial II (BHW)				
	n	**Neg[a]**	**Pv[b]**	**Pf[c]**	**M[d]**	***n***	**Neg**	**Pv**	**Pf**	**M**	**U[e]**	***n***	**Neg**	**Pv**	**Pf**	**M**
		227	**57**	**60**	**6**		**196**	**48**	**93**	**13**	**1**		**54**	**31**	**27**	**1**
Neg[a]	**149**	146	2	1	0	149	145	2	2	0	0	41	34	4	3	0
Pv[b]	**34**	1	32	1	0	34	1	32	1	0	0	34	8	25	1	0
Pf[c]	**167**	80	23	58	6	167	49	14	90	13	1	38	12	2	23	1

[a] Neg = no parasites.

[b] Pv = *Plasmodium vivax*.

[c] Pf = *Plasmodium falciparum*.

[d] M = mixed (Pf + Pv).

[e] U = undifferentiated *Plasmodium* species.

Concordance between ICT and microscopy in Trial I was significantly improved on re-reading the slides, from 76.0% to 84.6% for any species (χ^2 = 8.12, P = 0.004), and from 69.1% to 80.2% (χ^2 = 11.36, $P < 0.001$) for species-specific concordance. The concordance of the results obtained in Trial II, 76.1% for any species (χ^2 = 0.00, P = 0.98) and 73.5% for species-specific concordance (χ^2 = 0.76, P = 0.38) was similar to that in Trial I. Direct comparison with slides in Trial I indicated that the ICT test achieved a sensitivity of 97.2% (range 91.3–99.3%) and a specificity of 74.1% (range 68.0–71.4%) for specifying *P. falciparum*, and corresponding values of 91.4% (range 75.8–97.8%) and 98.9% (range 95.7–99.8%) for specifying *P. vivax* (excluding cases with both antigen bands positive).

Notable in these results is the high frequency of ICT-positive, slide-negative cases, which reduced considerably after re-reading in Trial I. All but one of these related to *P. falciparum* by ICT in Trial I (positive HRP-2 band), while 40% related to *P. vivax* in Trial II (only the P-S Ag band was positive).

Table 3 details the results of the re-read slides in Trial I. Of the 23 cases initially classified as *P. vivax* on slide but as *P. falciparum* by ICT (positive HRP-2), 14 were confirmed as *P. vivax*. For all the latter cases, P-S Ag was also detected, this being consistent with, but not specific for, *P. vivax*. One further case was confirmed as involving *P. falciparum* despite an ICT result specific for *P. vivax* (P-S Ag positive, HRP-2 negative). Of the 22 restained concordant negative results, only one was positive on re-reading.

After the Trial I slides had been re-read, many cases remained in which either the ICT result was a false positive or parasitaemias were missed by microscopy. Other characteristics of these discordant cases (ICT-positive, slide-negative) were therefore considered. The mean age of discordant cases in Trial I was similar to that of negative-negative cases (23.5 years), whereas confirmed positive cases were younger (mean = 14.1 years). The mean axillary temperatures were also similar (37.09 °C vs 37.05 °C, P = 0.73). However, the symptoms differed significantly, discordant cases reporting a greater frequency of symptoms than cases that were both ICT negative and slide negative. The difference increased as the range of symptoms increased (Table 4).

Comparisons were made between the HRP-2 and P-S Ag bands of the ICT tests and slide results. Of the cases that were positive by ICT and slide in Trial I, 94.7% had a positive P-S Ag band; the corresponding value for discordant cases was 48% (χ^2 = 6.26, P = 0.01). Only one discordant case had reported antimalarial therapy during the preceding month.

If only the P-S Ag band was considered, final concordance for any species between slide and ICT results in Trial I increased from 84.6% to 89.7% (χ^2 = 4.13, P = 0.04). The specificity of the ICT test, with microscopy as a standard, then improved from 74.5% (range 67.7–80.3%) to 87.7% (82.0–91.8%), the frequency of ICT-positive cases dropping from 25.6% of negative slides to 12.3% (χ^2 = 7.70, P = 0.006).

Discordant results in Trial I had a lower mean antigen band intensity than ICT-positive, slide-positive cases, suggesting lower parasite densities; both HRP-2 band intensities (F-statistic = 23.34, $P < 0.0001$) and P-S Ag band intensities (F-statistic = 72.30, $P < 0.0001$) were weaker. None of the 50 discordant cases had dark (grade 3) P-S Ag band intensities, whereas 44 of 151 positive-positive cases were in this category. Notwithstanding this trend in HRP-2 intensity, 16 of 49 ICT-positive, slide-negative *P. falciparum* cases had dark (grade 3) bands.

Methods for predicting parasitaemia

In view of the probable low actual false-positive rate given by ICT and microscopy, a positive result by ICT or slide was adopted for the purpose of subsequent analysis. The sensitivity and negative predictive value (NPV) of ICT and microscopy using this standard for patients with no recent history of treatment demonstrate the relatively low sensitivity of slides made and read in the conditions of the study (Table 5).

Table 3. **Trial I. Matched results of 129 slides read after transportation and storage under local conditions and re-read after restaining**

Local results	Slide results after restaining									
	n	**Neg[a]**		**Pv[b]**		**Pf[c]**		**M[d]**		**U[e]**	
n		**71**		**15**		**35**		**7**		**1**	
Neg	**103**	71	(21,1,49)[f]	0	–	30	(1,0,29)	1	(0,0,1)	1	(0,0,1)
Pv	**24**	0	–	15	(1,0,14)	3	(0,0,3)	6	(0,0,6)	0	–
Pf	**2**	0	–	0	–	2	(1,1,0)	0	–	0	–

[a–e] See footnotes [a–e], Table 2.

[f] Figures in parentheses are ICT test results: negative, P-S Ag positive, HRP-2 positive, respectively.

Table 4. **Symptoms reported by patients with no recent treatment in Trial I with positive ICT and positive slide results (I+/S+, *n* = 142), positive ICT and negative slide results (I+/S-, *n* = 49), and negative ICT and negative slide results (I-/S-, *n* = 139)[a]**

Symptom	**I+/S+ (%)**	**I+/S- (%)**	**I-/S- (%)**	**Odds ratio**		***P*-value[a]**
Fever (F)	96.0	94.0	82.8	3.26	(0.87–14.45[b])[c]	0.05
Chills (C)	87.4	88.0	60.7	4.75	(1.78–13.42[b])	<0.001
Headache (H)	74.8	94.0	82.1	3.42	(0.92–15.11[b])	0.04
F + C	84.1	84.0	52.4	4.77	(1.96–11.97)	<0.001
F + H	70.9	80.0	66.9	3.63	(1.35–10.30[b])	0.004
F + C + H	62.9	80.0	44.8	5.00	(2.15–11.52)	<0.001

[a] Odds ratio and *P*-values indicate probability of I+/S- patients having symptoms in comparison to I-/S patients.

[b] Upper limit may be inaccurate.

[c] Figures in parentheses are 95% confidence limits.

Table 5. **Trial I. comparison of prediction of parasitaemia by ICT, slide and reported symptoms in 334 patients, and axillary temperature in 244 patients with parasitaemia, using parasitaemia detected by ICT and/or slide as standard[a]**

Test/symptom	**Sensitivity (%)**		**Specificity (%)**		**PPV[b] (%)**		**NPV[c] (%)**	
ICT	97.9	(94.5–99.3)[d]	–	–	–	–	97.2	(92.5–99.1)
Slide (restained)	74.9	(68.1–80.7)	–	–	–	–	73.9	(66.9–79.9)
Slide (local)	60.0	(52.7–66.9)	–	–	–	–	64.1	(57.2–66.9)
Fever (F)	95.4	(91.1–97.7)	16.5	(11.0–24.0)	61.6	(55.8–67.1)	71.9	(53.0–85.6)
Chills (C)	86.2	(80.3–90.5)	38.8	(30.8–47.5)	66.4	(60.2–72.1)	66.7	(55.2–76.5)
Headache (H)	79.5	(73.0–84.8)	17.3	(11.6–24.8)	57.4	(51.3–63.3)	37.5	(26.0–50.5)
F + C	82.6	(76.3–87.5)	47.5	(39.0–56.1)	68.8	(62.4–74.6)	66.0	(55.8–75.0)
F + H	74.9	(68.1–80.7)	32.4	(24.8–40.9)	60.8	(54.3–67.0)	47.9	(37.6–58.4)
F + C + H	66.2	(59.0–72.7)	54.7	(46.0–63.1)	67.2	(60.0–73.7)	53.5	(45.0–61.9)
F + (C +/or H)	91.3	(86.2–94.7)	25.2	(18.4–33.4)	63.1	(57.2–68.7)	67.3	(52.8–79.3)
Axilliary temperature ⩾ 37.0 °C	67.5	(58.4–75.5)	45.8	(36.8–55.1)	56.1	(47.7–64.1)	57.9	(47.3–67.8)
Axilliary temperature ⩾ 37.5 °C	35.8	(27.5–45.0)	86.2	(78.7–91.4)	71.0	(57.9–81.4)	58.6	(51.3–65.6)
Axilliary temperature ⩾ 38.0 °C	23.6	(16.6–32.2)	93.3	(86.9–96.9)	78.4	(61.3–89.6)	54.4	(47.3–61.3)

[a] Recently treated patients excluded.

[b] PPV = positive predictive value.

[c] NPV = negative predictive value.

[d] Figures in parentheses are 95% confidence limits.

The sensitivity of diagnosis based on recent symptoms fell, and specificity rose, as the combination of symptoms increased (Table 5). Fever alone had a high sensitivity for predicting parasitaemia, but a very low specificity, and would result in 38.4% of patients receiving unnecessary treatment courses (1-positive predictive value) while failing to detect 4.6% of parasitaemias. Recent fever in combination with headache and/or chills, as used by the ADS-MCP algorithm, resulted in only a small improvement in specificity, while reducing sensitivity and missing 8.7% of parasitaemias. This would have resulted in 36.9% of patients receiving unnecessary treatment courses. A history of chills was more sensitive and specific than headache, but sensitivity and specificity remained low. Using all three symptoms lowered sensitivity below 70%, while still resulting in 32.8% of patients receiving unnecessary treatments and 46.5% of negative results being missed diagnoses (1-negative predictive value).

In Trial I the mean axillary temperature was significantly higher in patients with parasitaemia (37.39 °C vs 37.05 °C, Kruskall-Wallis $H = 10.45$, $P = 0.001$). Sensitivity, however, was very poor, and a majority of cases were missed where thresholds above 37.5 °C were used (Table 5).

Attitudes of barangay health workers to use of blood slides and ICT tests

Delayed slide results were considered of some use by 23 of the 27 BHWs questioned, although seven expressed reservations about late receipt. The most common perceived benefit, expressed by nine respondents, was the determination of *P. vivax* cases to be treated with primaquine. The results were used for patient follow-up, as a patient education tool (five respondents), and, if positive, for retreatment (against the treatment protocol) (four respondents). Other perceived benefits included self-education and the demonstration of the performance of their duties.

All 27 BHWs considered that it was advantageous to obtain immediate diagnosis by means of the ICT tests. Five modified their normal practice by including closer follow-up of positive cases and three did so by either withholding treatment or withdrawing treatment because of side-effects after negative results. Three searched harder for alternative diagnoses after negative results. Seven BHWs mentioned that a preference among patients for ICT tests led to increased treatment-seeking behaviour and compliance. Some mentioned increases in job satisfaction, their standing in the community, and their clinical knowledge.

Community interviews

Seventy-two adults from four villages gave responses to the three alternative diagnostic strategies. One respondent would not use any intervention based on the health service. The answers of the remaining 71 are given in Table 6. Most (63%) preferred investigation that involved some form of blood sampling. Rapid diagnostic tests were easily the most popular choice, despite the cost, which was roughly half the minimum true cost of the tests. Delayed slide-based diagnosis was preferred to unconfirmed symptom-based diagnosis.

Discussion

The reduction of morbidity and the interruption of parasite transmission by means of community-based antimalarial treatment requires an accurate, rapid and practical method of remote diagnosis. Assessing the most appropriate diagnostic option is limited by difficulties in comparing one imperfect test with another; in this case symptom-based diagnosis, microscopy and a rapid diagnostic test. A balance has to be struck between avoiding missed diagnoses, with consequences for the health of individuals and populations through continued transmission, and minimizing unnecessary treatment. The high rate of afebrile parasitaemias in this study indicated that a large reservoir of barely symptomatic cases existed in the community. A sustained treatment effort involving the use of a highly sensitive diagnostic method to detect new cases is needed while this infectious reservoir is eliminated by natural clearance.

In the remote area of the study the accuracy of symptom-based diagnosis was poor, as it has been elsewhere (*6*, *7*), although specificity in this self-referring sick population should be higher than in the community as a whole. Local transmission rates, and therefore immunity, were low, and this should have increased the sensitivity (*24, 25*). The observation of fever alone, and of fever in combination with chills and/or headache, achieved quite high sensitivities, but both criteria resulted in high rates of over-treatment. Any narrower combination of symptoms resulted in sensitivities unacceptable in relation to the detection of a life-threatening illness. The measurement of axillary temperature failed to achieve sufficient sensitivity or specificity to be useful.

The ICT tests prepared by experienced personnel in Trial I demonstrated good sensitivity and negative predictive values in comparison with locally read slides and the slides restained and read in a more controlled environment. Specificity and positive predictive values for the ICT tests are difficult to assess when comparison is made with microscopy as a standard, which itself has poor sensitivity at low parasite densities (*26*, *27*). They were probably much higher than this study indicated, for the following reasons.

First, the greater concordance between slides and ICT P-S Ag than between slides and ICT HRP-2 is consistent with the shorter half-life of the P-S Ag in the circulation. Many patients would have been infected for some time, as little treatment had been available previously, and low fluctuating parasite densities in this situation are only intermittently detectable by microscopy (*24*, *26*). HRP-2 is detectable for a week or more after microscopically patent

parasitaemia (*28*, *29*), while P-S Ag rapidly becomes undetectable (*20*) and more closely reflects the results of microscopic investigation. The reduced intensity of both antigen bands in ICT-positive, slide-negative (discordant) results in comparison with positive-positive cases is consistent with a reduced parasite density in this group (*17*, *20*).

Second, it is unlikely that a significant number of false-positive ICT results in Trial I occurred because of persistent antigen after the resolution of parasitaemia, as little treatment had been available. Mean parasitaemic durations for *P. falciparum* commonly extend beyond four months (*24*, *25*), so only a small proportion of positive cases could have resolved naturally just before sampling.

Third, the significantly greater incidence of symptoms in discordant cases relative to negative-negative cases suggests that two separate populations were involved. The discordant group is more likely to have been parasitaemic. The absence of a higher temperature in this group is not surprising, given the poor correlation between temperature and parasitaemia. The generally older age of discordant cases may reflect higher immunity-reducing parasite densities and the sensitivity of microscopy. Possible cross-reactivity of the HRP-2 band with rheumatoid factor (*30, 31*) does not explain the association with symptoms in this study, and cross-reactivity with other pathogens or acute-phase reactants has not been recorded.

Fourth, specificity for the HRP-2 band using microscopy as a standard has been much higher in other studies with different populations and treatment availabilities (*13*, *17*, *18*, *32*). The specificity of ICT tests would be higher early in the course of infection when higher parasitaemias improve microscopic sensitivity.

In view of the above, the 14 cases for which both antigen bands were positive by ICT (i.e. *P. falciparum* or mixed) but for which only *P. vivax* was indicated on slides are likely to have been mixed infections with a low fluctuating *P. falciparum* parasitaemia that was missed by microscopy. The P-S Ag band was more sensitive for the detection of *P. vivax* than in previous studies (*11*, *20*). This may be explained by the apparent high *P. vivax* parasite densities in the area, as all 33 *P. vivax* cases from three recent surveys had parasite densities greater than 450 per μl (Agusan del Sur Malaria Control Programme, unpublished data; Bell et al., unpublished data).

The similarity in ICT test results and slide concordance between the two trials indicates that the BHWs were achieving ICT results of similar accuracy to those of the researchers, despite minimal training and no supervision, and notwithstanding the storage of the tests at relatively high ambient temperatures. The ICT tests appear to have been considerably more accurate than the BHWs' slides, although further investigation is needed into the reduction in band intensity after prolonged storage.

Table 6. **Preferences of 71 community members for diagnostic methods in cases of fever**

	Slide	RDT[a] at cost of P 25[b]	Symptom only
First choice	11	34	26
Second choice	46	19	6
Third choice	14	18	39
Combined score[c]	**68**	**87**	**58**

[a] RDT = Rapid diagnostic test.

[b] P 25 = US$ 0.60.

[c] First choice = 2; second choice = 1; third choice = 0.

The ability to provide an immediate diagnosis using ICT tests allowed the BHWs to vary patient management according to the results. Thus their time could be more appropriately allocated. This ability also encouraged a search for alternative diagnoses after a negative result, and job satisfaction was apparently improved by the ability to provide a technical service previously reserved for professionals.

If, as the evidence suggests, many discordant cases were parasitaemic, the poor sensitivity obtained by microscopy in these conditions is of concern if slides are to be used as a basis for determining treatment or for planning purposes. Despite their limited value, microscopy results received after treatment were preferred by BHWs and community members to reliance on symptoms alone. Blood sampling was thought to enhance BHW status or credibility, and the results acted as a catalyst for further contact with patients and for education.

Community attitudes to diagnosis demonstrated the importance of providing patients with a reliable explanation for their illness. The BHWs suggested that this improved treatment-seeking behaviour and compliance. An immediate blood-based diagnosis at some cost was preferred by community members to both delayed free slide diagnosis and symptom-based diagnosis, despite the cost to the patient. This cost, approximately the difference between the estimated cost of microscopy to the health service and the bulk wholesale cost of the rapid diagnostic tests, was a substantial sum to families engaged in semisubsistence agriculture.

The cost-effectiveness of symptom-based diagnosis, rapid diagnostic tests and microscopy, and the proportion of costs that the community is willing to bear need further investigation. They can be expected to vary with transmission rates and health-service access. In the long term, improved compliance and treatment-seeking behaviour may bring additional economic benefits from rapid diagnostic tests through a reduced burden of illness. The detection of persistent antigen in asymptomatic infection, when fluctuating parasitaemia reduces the sensitivity of microscopy (*24*, *26*), also offers new possibilities for rapid screening of communities at risk.

This study clearly shows that malarial parasitaemia cannot be easily identified by symptoms alone and that microscopy is unreliable in remote areas. The rapid diagnostic test was well accepted by community volunteers and was performed accurately by them after little training. It markedly improved diagnostic accuracy and met a desire in the community for rapid blood-based diagnosis. ■

Acknowledgements

We are grateful for the active support and encouragement of the BHWs, officials and community members of the barangays of Angeles, Bataan, Langasian, Milagros, Remedios and Salug. We thank the RHU staff of Esperanza and La Paz, Agusan del Sur, together with the Malaria Control Service and the staff of ADS-MCP in Agusan del Sur and at the Research Institute for Tropical Medicine, Manila. The project received funding from the Australian National Health and Medical Research Council. ADS-MCP is funded by AusAID.

Conflicts of interest: none declared.

Résumé

Diagnostic du paludisme dans une région reculée des Philippines : comparaison de plusieurs techniques et de leur acceptation par des agents de santé et par la communauté

Objectif Comparer l'efficacité du diagnostic du paludisme d'après les symptômes, au moyen de tests rapides ou par examen microscopique dans une région reculée des Philippines où la maladie est faiblement endémique.

Méthodes Lors de l'essai I, des tests immunochromatographiques (ICT Malaria Pf/Pv) et des frottis sanguins ont été réalisés dans les villages sur 350 patients symptomatiques. Les lames ont été conservées et lues dans les conditions locales habituelles, puis ultérieurement soumises à une nouvelle coloration et une nouvelle lecture. Lors de l'essai II, des agents de santé de *barangay* volontaires et non supervisés ont préparé les tests ICT et les frottis après avoir reçu une brève formation. Les lames étaient lues dans les centres de santé ruraux. Par la suite, 27 agents de santé de *barangay* et 72 membres de la communauté ont été interrogés au sujet des trois stratégies de diagnostic.

Résultats La fièvre considérée isolément était sensible (95,4 %) mais peu spécifique (16,5 %) en tant que facteur prédictif de parasitémie. Lorsqu'on ajoutait d'autres symptômes, la sensibilité tombait au-dessous de 85 % mais la spécificité restait faible. La température axillaire n'avait qu'une faible valeur prédictive. Les tests ICT avaient une bonne sensibilité (97,9 %) mais de nombreux cas signalés comme positifs par le test étaient négatifs à l'examen microscopique. Une analyse plus poussée de ces cas lors de l'essai I a montré que les tests ICT détectaient des parasitémies faibles qui étaient passées inaperçues à l'examen microscopique et que ce dernier, effectué localement, était peu exact. Les tests ICT étaient bien acceptés et correctement réalisés par les agents de santé de *barangay*.

Conclusion Ces tests répondent au désir de la communauté de bénéficier d'un diagnostic réalisé sur prélèvement sanguin, sont susceptibles d'améliorer l'observance et peuvent modifier le comportement des patients en les amenant davantage à consulter.

Resumen

Diagnóstico del paludismo en una zona remota de Filipinas: comparación de distintas técnicas y de su aceptación por los agentes de salud y por la comunidad

Objetivo Comparar la eficacia, en una zona de Filipinas de baja endemicidad del paludismo, de tres sistemas de diagnóstico de esta enfermedad: un sistema de diagnostico remoto basado en los síntomas, pruebas diagnósticas rápidas, y el diagnóstico microscópico.

Métodos En el Ensayo I se analizó a 350 pacientes sintomáticos mediante pruebas de inmunocromatografía (ICT) Malaria Pf/Pv y extensiones sanguíneas almacenadas y analizadas en condiciones locales. Las extensiones se volvieron a teñir y analizar más adelante. En el Ensayo II, agentes de salud de *barangay* voluntarios prepararon sin supervisión pruebas ICT y extensiones tras una breve capacitación; las extensiones fueron analizadas en unidades de salud rurales. Posteriormente se interrogó a 27 agentes de salud de *barangay* y a 72 miembros de la comunidad acerca de las tres estrategias diagnósticas.

Resultados La presencia de fiebre sin otras manifestaciones resultó ser un criterio sensible (95,4%) pero poco específico (16,5%) para predecir la parasitemia. La inclusión de otros síntomas redujo la sensibilidad a niveles inferiores al 85%, pero la especificidad siguió siendo baja. La temperatura axilar tenía poco valor predictivo. Las pruebas de ICT demostraron tener una alta sensibilidad (97,9%), pero muchos casos positivos según esas pruebas fueron también negativos al análisis microscópico. El posterior análisis de esos casos en el Ensayo I mostró que las pruebas de ICT estaban detectando parasitemias bajas no detectadas mediante la microscopía, y que esta última técnica, aplicada localmente, era poco precisa. Las pruebas de ICT fueron bien aceptadas y correctamente realizadas por los agentes de salud de *barangay*.

Conclusión Estas pruebas vienen a cubrir la apremiante necesidad que tiene la comunidad de diagnósticos sanguíneos y pueden favorecer la observancia y la búsqueda de tratamiento por los pacientes.

References

1. **Nabarro DN, Tayler EM.** The ''Roll Back Malaria'' campaign. *Science*, 1998, **280**: 2067–2068.
2. **Krogstad DJ, Ruebush TK.** Community participation in the control of tropical diseases. *Acta Tropica*, 1996, **61**: 77–78.
3. **Pagnoni F et al.** A community-based programme to provide prompt and adequate treatment of presumptive malaria in children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1997, **91**: 512–517.
4. **Goodman CA, Coleman PG, Mills AJ.** Cost-effectiveness of malaria control in sub-Saharan Africa. *Lancet*, 1999, **354**: 378–385.
5. *Roll Back Malaria. A global partnership.* Geneva, World Health Organization (Internet communication at http://www.who.int/rbm/about.html).
6. **Armstrong-Schellenberg JRM et al.** What is clinical malaria? Finding case definitions for field research in highly endemic areas. *Parasitology Today*, 1994, **10**: 439–442.
7. **Bruce-Chwatt LJ.** *Essential malariology*, 2nd ed. London, Heinemann, 1985.
8. **Ghebreyesus TA et al.** Pilot studies on the possible effects on malaria of small-scale irrigation dams in Tigray regional state, Ethiopia. *Journal of Public Health Medicine*, 1998, **20**: 238–240.
9. **Okanurak K, Ruebush TK.** Village-based diagnosis and treatment of malaria. *Acta Tropica*, 1996, **61**: 157–167.
10. **Ruebush TK et al.** Use of illiterate volunteer workers for malaria case detection and treatment. *Annals of Tropical Medicine and Parasitology*, 1990, **84**: 119–125.
11. **Tjitra E et al.** Field evaluation of the ICT malaria P.f/P.v immunochromatographic test for detection of *Plasmodium falciparum* and *Plasmodium vivax* in patients with a presumptive clinical diagnosis of malaria in eastern Indonesia. *Journal of Clinical Microbiology*, 1999, **37**: 2412–2417.
12. **Bojang KA.** The diagnosis of *Plasmodium falciparum* infection in Gambian children, by field staff using the rapid, manual, ParaSight™-F test. *Annals of Tropical Medicine and Parasitology*, 1999, **93**: 685–687.
13. **Singh N, Valecha N, Sharma VP.** Malaria diagnosis by field workers using an immunochromatographic test. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1997, **91**: 396–397.
14. **Premji Z, Minjas JN, Shiff CJ.** Laboratory diagnosis of malaria by village health workers using the rapid manual ParaSight-F test. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1994, **88**: 418.
15. **Mharakurwa S, Manyame B, Shiff CJ.** Trial of the ParaSight-F test for malaria diagnosis in the primary health care system, Zimbabwe. *Tropical Medicine and International Health*, 1997, **2**: 544–550.
16. **Singh N, Singh MP, Sharma VP.** The use of a dipstick antigen-capture assay for the diagnosis of *Plasmodium falciparum* infection in a remote forested area of central India. *American Journal of Tropical Medicine and Hygiene*, 1997, **56**: 188–191.
17. **Kumar A et al.** Clinical trials of a new immunochromatographic test for diagnosis of *Plasmodium falciparum* malaria in Goa. *Indian Journal of Malariology*, 1996, **33**: 166–172.
18. **Thepsamarn P et al.** The ICT Malaria Pf: a simple, rapid dipstick test for the diagnosis of *Plasmodium falciparum* malaria at the Thai-Myanmar border. *Southeast Asian Journal of Tropical Medicine and Public Health*, 1997, **28**: 723–726.
19. **Banchongaksorn T et al.** Operational trial of ParaSight-F (dipstick) in the diagnosis of *falciparum* malaria at the primary health care level. *Southeast Asian Journal of Tropical Medicine and Public Health*, 1997, **28**: 243–246.
20. **Eisen DP, Saul A.** Disappearance of pan-malarial antigen reactivity using the ICT Malaria P.f/P.v™ kit parallels decline of patent parasitaemia as shown by microscopy. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2000, **94**: 169–170.
21. **Field JW, Sandosham AA, Yap LF.** Technical miscellanea. *The microscopical diagnosis of human malaria. I. A morphological study of erythrocytic parasites in thick blood films*, 2nd ed. Kuala Lumpur, Institute of Medical Research, 1963: 46–83.
22. **Russell PF et al.** Laboratory and field techniques. In: *Practical malariology*, 2nd ed. London, Oxford University Press. 1963: 132–133.
23. **Dean AG et al.** *Epi Info, Version 6: a word-processing, database and statistics program for public health on IBM-compatible microcomputers*. Centers for Disease Control and Prevention, Atlanta, GA, 1997.
24. **Collins WE, Jeffery GM.** A retrospective examination of sporozoite- and trophozoite-induced infections with *Plasmodium falciparum*: development of parasitologic and clinical immunity during primary infection. *American Journal of Tropical Medicine and Hygiene*, 1999, **61**: 4–19.
25. **Powell RD, McNamara JV, Rieckmann KH.** Clinical aspects of acquisition of immunity to *falciparum* malaria. *Proceedings of the Helminthological Society of Washington*, 1972, **39**: 51–66.
26. **Roper C et al.** Detection of very low level *Plasmodium falciparum* infections using the nested polymerase chain reaction and a reassessment of the epidemiology of unstable malaria in Sudan. *American Journal of Tropical Medicine and Hygiene*, 1996, **54**: 325–331.
27. **Tham JM et al.** Detection and species determination of malaria parasites by PCR: comparison with microscopy and with ParaSight-F and ICT malaria Pf tests in a clinical environment. *Journal of Clinical Microbiology*, 1999, **37**: 1269–1273.
28. **Mharakurwa S, Shiff CJ.** Post treatment sensitivity studies with the ParaSight-F test for malaria diagnosis in Zimbabwe. *Acta Tropica*, 1997, **66**: 61–67.
29. **Shiff CJ, Premji Z, Minjas JN.** The rapid manual ParaSight-F test. A new diagnostic tool for *Plasmodium falciparum* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1993, **87**: 646–648.
30. **Iqbal J, Sher A, Rab A.** *Plasmodium falciparum* histidine-rich protein 2-based immunocapture diagnostic assay for malaria: cross-reactivity with rheumatoid factors. *Journal of Clinical Microbiology*, 2000, **38**: 1184–1186.
31. **Grobusch H, Jelinek T, Hänscheid T.** False positivity of rapid antigen detection tests for diagnosis of *Plasmodium falciparum* malaria: issue appears to be more complicated than presented. *Journal of Clinical Microbiology*, 1999, **37**: 3781–3782.
32. **Stow NW, Torrens JK, Walker J.** An assessment of the accuracy of clinical diagnosis, local microscopy and a rapid immunochromatographic card test in comparison with expert microscopy in the diagnosis of malaria in rural Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1999, **93**: 519–520.