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MalaQuick™ versus *Para*Sight F® as a diagnostic aid in travellers' malaria

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Abstract

In this study we assessed whether travellers can perform malaria rapid tests, following the provided information leaflet, and correctly interpret performed and pre-prepared test strips. Two *Plasmodium falciparum* testing systems, namely MalaQuick™ (ICT) and *Para*Sight F® were used. Test performance and test interpretation of pre-prepared tests were compared. There was no significant difference in test performance between the 2 tests. Interpretation of prepared test strips in both test systems was very reliable in blood parasite densities between 0·1% and 2%, but major problems were encountered at low parasitaemia (< 0·1% blood parasites) and also in *Para*Sight F test strips showing high parasitaemia (> 2% blood parasites). Low parasitaemia *Para*Sight F test strips were correctly interpreted by 52·1% compared with 10·8% correct interpretations with MalaQuick ($P < 0·0001$). Correct interpretation of highly positive (> 2% blood parasites) pre-prepared test strips was higher with MalaQuick (96·8%) than with *Para*Sight F (33·8%), $P < 0·0001$. Both tests were associated with high levels of false-negative interpretations which render them unsuitable as self-diagnostic kits. Efforts must be made to assist lay individuals in test performance by technical test improvement, by equiping the test strips with an additional reading aid for interpretation, and by providing instruction by a skilled person.

Keywords: malaria, diagnosis, rapid test, *Para*Sight F®, MalaQuick™ (ICT), travellers

Introduction

Travel to tropical countries shows a continuous annual growth and malaria infection is a permanent threat at many destinations. Each year some 10 000 travellers residing in non-endemic countries are reported to import malaria and this figure is considered to be an underestimate (MUENTENER *et al.*, 1999). In travellers, malaria diagnosis is often made only after return to the home countries, and confirmed by trained and skilled laboratory technicians performing a microscope examination of a Giemsa-stained blood smear. The skills and technical equipment in developing countries may in some cases be suboptimal or unavailable. Malaria, even severe malaria, is often not easily distinguishable from other diseases (BASSET *et al.*, 1991; LUXEMBURGER *et al.*, 1998). The case–fatality rate of malaria in tourists may rise to over 3%, even if the diagnosis is made in industrialized countries (MUENTENER *et al.*, 1999). Stand-by emergency treatment for self-administration is often recommended for certain traveller groups including travellers to low-endemicity areas or isolated locations far from competent medical services (SCHLAGENHAUF & STEFFEN, 1994). Self-diagnosis of malaria is difficult under such conditions and incorrect treatment of malaria is associated with unnecessary adverse events or delayed, inappropriate use of the therapy (SCHLAGENHAUF *et al.*, 1995). It has been suggested that an easily performed, reliable malaria diagnostic test kit could be very important for travellers, to support the stand-by treatment strategy (SCHLAGENHAUF *et al.*, 1995).

In 1991, PARRA *et al.* demonstrated the presence of *Plasmodium falciparum* histidine-rich protein 2 (PfHRP-2) (HOWARD *et al.*, 1986) in the plasma of humans with *P. falciparum* malaria. The identification of this protein in humans with malaria led to the development of non-microscopy tests, demonstrating the infection with *P. falciparum* by means of an immunological capture assay. The paper test strip has been proven to be sensitive, specific and easy to perform and does not require electricity or equipment. Only a small amount of unprocessed blood is needed. The result can be read with the naked eye and the components are stable under warm conditions (SHIFF *et al.*, 1993). In 1994 BEADLE *et al.* examined the test in a field trial in Kenya. Sensitivity and specificity were good in high-grade parasitaemias, but decreased markedly in lower-grade parasitaemias. The value of such tests performed by instructed staff for the diagnosis of falciparum malaria has been shown in several studies of commercially available test kits, e.g., the *Para*Sight F® test (DIETZE *et al.*, 1995; BANCHONGAKSORN *et al.*, 1997; HUMAR *et al.*, 1997; KODISINGHE *et al.*, 1997; MHARAKURWA *et al.*, 1997; SINGH *et al.*, 1997a), and ICT Malaria Pf = MalaQuick™ test (KUMAR, 1996; SINGH *et al.*, 1997b; VALECHA *et al.*, 1998). All studies confirmed the usefulness of the rapid detection test kits when performed by skilled laboratory technicians who are usually experienced in performing different immunological tests. These tests detect only *P. falciparum* parasites, which are however responsible for > 50% of all imported malaria cases and for the majority of life-threatening malaria infections. The value of the currently used tests is restricted to areas with predominant *P. falciparum* infection (UGUEN, 1995). In 1996 TRACHSLER *et al.* (1999) tested the Becton Dickinson Malaria kit, the *Para*Sight F test, prospectively in travellers, but the test did not prove to be suitable for travellers' self-diagnosis of malaria. To evaluate the MalaQuick test an additional study has been performed with identical methods. The results of these 2 analyses are compared in this report.

Material and Methods

Subjects

Healthy travellers aged 20–39 years with destinations to areas with low-endemicity for malaria were recruited at our travel clinic in Zurich, Switzerland. Older age-groups originally recruited had to be excluded from the analysis, because of statistically significant differences in age, sex or education.

A group of 93 volunteers performed the MalaQuick test between October 1997 and February 1998. This group was compared with 71 volunteers who performed the *Para*Sight F test in a comparable study in 1996 (TRACHSLER *et al.*, 1999). The educational level of subjects in each study was high with 40·9% at university level or equivalent. The 2 study groups were comparable with regard to sex and educational status.

Methods

The objective of the study was to assess whether a lay person can perform and interpret a commercially available malaria test following the provided written instructions only.

The study was approved by the University of Zurich Ethics Committee. The goal was explained to the volunteers before they signed the informed consent form.

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Volunteers were asked to perform a rapid malaria test using their own blood according to the provided instruction leaflet. They also completed a questionnaire and interpreted 5 test strips previously prepared by skilled laboratory staff from clinically obtained blood specimens with different parasite densities. Study procedures in both study populations were identical or adapted for different techniques. The pre-prepared test strips, 'low' (< 0·1%), 'intermediate' (0·1–2%) and 'high' (> 2%) parasitaemia, together with 'negative' and 'control' were the same for each traveller in each of the 2 groups and they were prepared according to the Giemsa-stained film examination of the same blood. They were provided by trained laboratory staff at the Swiss Tropical Institute in Basel, Switzerland. The 'control' test strip was run with 1 drop of buffer and no control line appeared. Test performance, questionnaire completion and test interpretation of the pre-prepared strips were done immediately at the travel clinic. Medical professionals were excluded.

Materials

MalaQuick tests (ICT Malaria Pf test) and written test instructions (in German) were provided by Standby Diagnostics, Munich, Germany. One drop (~ 10 µL) of whole blood is applied directly on a testcard with a paper-like strip imbedded with 2 antibodies specific for PfHRP-2, one attached to visible colloidal gold and impregnated in the sample pad, the other immobilized in a line across the strip. If PfHRP-2 is present in the plasma, it binds to the gold-labelled antibody. It is then run with the buffer (reagent A) and will be captured by the membrane-attached second antibody, forming a pink line. A control line appears above this line confirming the validity of the test. Only 10 µL of blood are required.

*Para*Sight F tests and written test instructions (in German) were provided by Becton Dickinson, Cockeysville, MD, USA. The dipstick, a preparation of nitrocellulose and glass fibre is pretreated with a mouse monoclonal antibody (mAb) against PfHRP-2 which is applied in a line about 1 cm from the base of the dipstick. A second dotted line of PfHRP-2 antigen is incorporated in the dipstick about 2–3 mm above the line of mAb as a reagent control. To perform the test 50 µL of whole blood measured in a capillary glass tube are required. The procedure has been previously described in detail (TRACHSLER *et al.*, 1999).

A questionnaire, targeting the assessments, use of the diagnostics, interpretation and acceptability of the tests was completed by each volunteer. It was designed to pinpoint areas of difficulty in the test procedures.

The data were analysed using SSPS and significance was determined using the χ^2 test with $P < 0.05$ considered to be significant.

Results

Performance of self-testing

The procedure of self-testing showed no significant differences between the 2 tests (Fig. 1): 87·1% of the MalaQuick subjects and 74·6% of the *Para*Sight F subjects performed the tests correctly. Test performance and rating with regards to level of difficulty is shown in Figure 2.

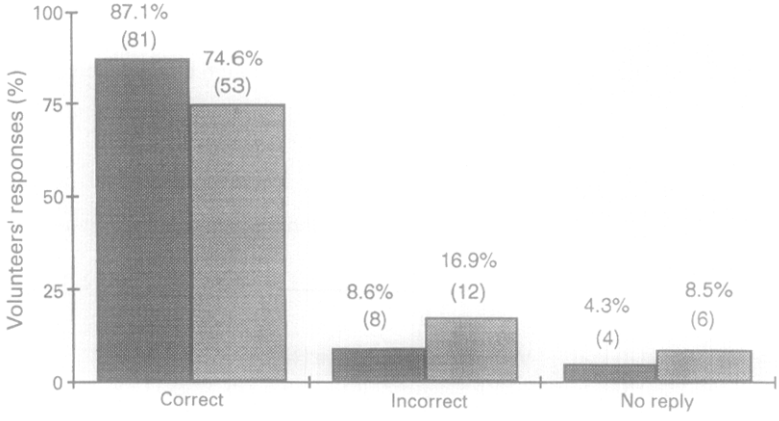

Fig. 1. *Para*Sight F® versus MalaQuick™: accuracy of test performance. Dark columns, MalaQuick ($n = 93$); paler columns, *Para*Sight F ($n = 71$).

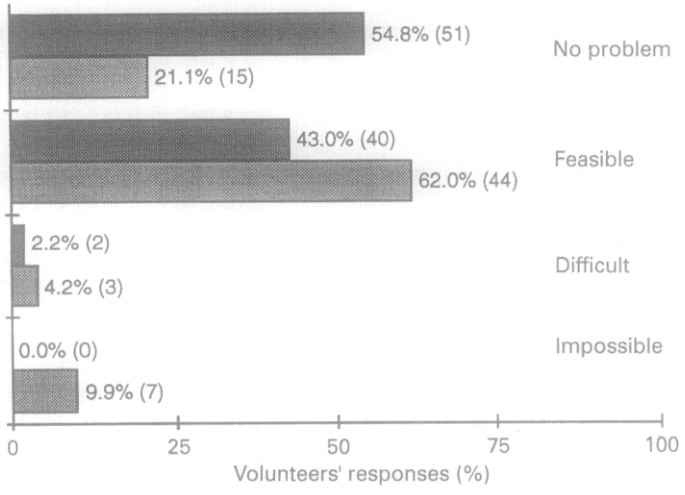

Fig. 2. *Para*Sight F® versus MalaQuick™: difficulty rating for test procedures. Dark columns, MalaQuick ($n = 93$); paler columns, *Para*Sight F ($n = 71$).

The MalaQuick test was significantly easier to perform ($P < 0.0007$). No reply to this question was given by 2 (2·8%) persons in the *Para*Sight F group.

Test instructions

These were considered to be concise and clear by 64 (68·8%) individuals of the MalaQuick group and by 46 (64·8%) volunteers in the *Para*Sight F group, but identification of test components was a problem for many participants. Some 43 (46·2%) of the MalaQuick and 33 (46·5%) of *Para*Sight F participants did not understand the meaning of specific terms.

There were significantly ($P = 0.003$) fewer requests for additional test instructions in the MalaQuick group compared to the *Para*Sight F group. Some 65 (69·9%) of the MalaQuick and 32 (45·1%) of the *Para*Sight F subjects considered additional information to be 'superfluous'. There was no distinct wish in either group to see a video demonstration before performing the test.

Blood collection

Blood collection was considered to be more difficult ($P = 0.001$) in the *Para*Sight F group as it was rated as 'difficult' by 48 (67·6%) subjects versus 40 (43%) in the MalaQuick group (Fig. 3).

Test development

There were no major differences between the groups regarding test development. The *Para*Sight F test procedure is more time consuming than that of the MalaQuick test ($P < 0.0001$). The majority (40, 56·3%) of *Para*Sight F users needed 15–30 min for the test performance, whereas almost all (89, 95·7%) of MalaQuick users did the test in less than 15 min.

Interpretation of pre-prepared test strips (Table)

Test interpretation at parasite levels of 0·1–2% did not differ significantly in the 2 tests. Low parasitaemia of < 0·1% parasites was more accurately interpreted with *Para*Sight F ($P < 0.0001$) whereas high parasitaemia showed a higher accuracy ($P < 0.0001$) with MalaQuick. Pre-prepared 'negative' test strips (i.e., no parasitaemia) were also better recognized by MalaQuick volunteers ($P = 0.014$).

User acceptability rating

There was a high level of acceptance for both tests. Only 1 (1·4%) *Para*Sight F and 0 (0%) MalaQuick users felt that such tests were 'superfluous'. Some 35 (37·6%) MalaQuick and 17 (23·9%) *Para*Sight F volunteers considered the tests as 'indispensable' for travellers'

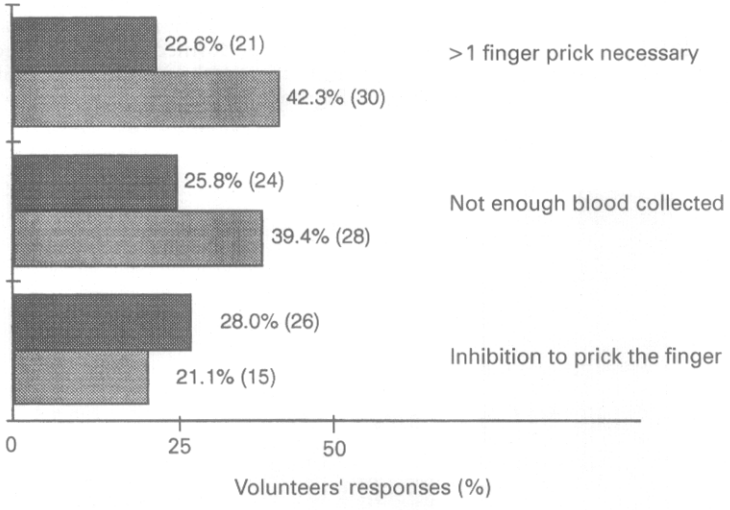

Fig. 3. *Para*Sight F® versus MalaQuick™: problems in blood collection. Dark columns, MalaQuick ($n = 93$); paler columns, *Para*Sight F ($n = 71$).

Table. *Para*Sight F® versus MalaQuick™: test interpretation of prepared test strips

Parasite level	Positive	Negative	No result	Uncertain	No answer
0·1–2% (positive)[a]					
*Para*Sight F	**65 (91·5%)**	6 (8·5%)	0	0	1 (1·4%)
MalaQuick	**89 (95·7%)**	1 (1·1%)	2 (2·2%)	1 (1·1%)	0
< 0·1% (positive)[a]					
*Para*Sight F	**37 (52·1%)**	21 (29·6%)	6 (8·5%)	4 (5·6%)	3 (4·2%)
MalaQuick	**10 (10·8%)**	67 (72·0%)	2 (2·2%)	14 (15·1%)	0
0 (negative)[a]					
*Para*Sight F	0	**57 (80·3%)**	6 (8·5%)	5 (7·0%)	3 (3·2%)
MalaQuick	3 (3·2)	**88 (94·6%)**	1 (1·1%)	1 (1·1%)	0
> 2% (positive)[a]					
*Para*Sight F	**24 (33·8%)**	19 (26·8%)	15 (21·1%)	10 (14·1%)	3 (3·2%)
MalaQuick	**90 (96·8%)**	3 (3·2%)	0	0	0
– (control)					
*Para*Sight F	0	5 (7·0%)	**61 (85·9%)**	2 (2·8%)	3 (4·2%)
MalaQuick	1 (1·1%)	0	**92 (98·9%)**	0	0

The correct interpretation is given in **bold** numbers.
[a]Expected test results.
The 'control' test strip was run with one drop of buffer, no control line appeared.

needs. The majority of both study populations [53 (74·6%) of the *Para*Sight F group and 57 (61·3%) of the MalaQuick group] regarded the test as helpful.

Discussion

The general difficulties that lay-persons encounter in performing laboratory tests are clearly demonstrated in this study. The accuracy of test performance was high, but not high enough. Some practical difficulties such as blood collection or the lack of experience are unavoidable. In an emergency situation, it is doubtful whether a febrile, unwell patient could follow the instructions and interpret the test correctly even if the need to perform the test were great. A drawback with the *Para*Sight F test is the larger amount (50 μL) of blood required for successful test performance whereas in the MalaQuick test a single droplet (~ 10 μL) suffices. With both tests the identification of test components for lay-persons is a problem and these must be more clearly labelled to facilitate identification and handling of the components. Efforts have also to be made to reduce areas of difficulty such as fingerpricking and to increase the ease of the test performance by technical means or different optical design. For both tests, it is apparent that the test procedures must be modified to be practical in daily routine situations for technically untrained persons.

A further problem, which has not been previously mentioned, is that the control line indicates that the test procedure was correct but not that blood was actually tested. Without testing one single drop of blood there will be a control line. Therefore an additional control should be added marking a human blood protein and demonstrating that the test has been correctly run with blood and buffer. This is particularly important in the MalaQuick procedure, in which no defined quantity of blood is tested.

Test interpretation is an important problem that has not been resolved in these tests. Interpretation of parasite densities of 0·1–2% is accurate in both test systems. The most important limitation of the tests is poor interpretation at low (both tests) and high parasitaemia levels (*Para*Sight F). In locations with poor lighting, the results may be even worse, especially for elderly people with naturally occurring presbyopia or in sick persons with possibly impaired vision. Low parasitaemias are most common in travellers. False-negative interpretation in low parasitaemia of up to 72·0% (MalaQuick) and 29·6% (*Para*Sight F) is unacceptable. Interpretation of high parasitaemia was an additional problem in the *Para*Sight F group, which renders the test unsuitable in cases of severe parasitaemia. False-positive interpretations were not a major problem, but could lead to the inappropriate use of malaria treatment with possible concomitant adverse effects or may also lead to delayed diagnosis and inappropriate treatment of other important causes of fever such as typhoid or dengue fever. Recent literature reports have identified an additional problem of possible false-positive test results in the presence of rheumatoid factors as shown by GROBUSCH *et al.* (1999) and BORTOLONI *et al.* (1998).

Test interpretation needs additional clarification and in-built controls, perhaps by use of reading aids and clearer instruction with printed samples of test results that can be compared for correct interpretation. This could simplify the diagnostic procedure for lay-persons and make additional oral instruction superfluous.

Medical testing systems should be evaluated for lay-persons before being sold over the counter and providers of such tests should be skilled instructors. The hazard of selling a testing system to lay-persons, thus giving a false sense of security, must be avoided until such tests are fool-proof. The very high user acceptability rate strongly suggests that efforts should be made to improve and simplify the test performance and interpretation.

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Short Report

Growth and maturation of *Plasmodium falciparum* in BacT/Alert blood culture system

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Keywords: blood cultures, BacT/Alert, malaria, diagnosis

The BacT/Alert microbial detection system (Organon Teknika Corporation, Durham, NC) is used internationally for culture of bacterial pathogens from blood. Aerobic and anaerobic vials contain supplemented tryptic soy broth with additional complex amino acid and carbohydrate substrates in an atmosphere of CO_2 in air (aerobic) or nitrogen (anaerobic) under vacuum. Sodium polyanetholesulfonate (0·035%) is used as an anticoagulant. Each vial is inoculated with 5–10 mL of blood. If microorganisms are present in the test sample, they metabolize the substrate in the culture medium and CO_2 is produced. The CO_2 diffuses through a selectively permeable membrane and into a CO_2 sensor at the base of the bottle. Free hydrogen ions generated by the interaction of CO_2 with H_2O change the colour of the sensor from dark green to yellow. A light-emitting diode (LED) projects light on to the sensor. The reflected light is measured by a photo detector. As more CO_2 is generated, more light is reflected. This information is transmitted to a computer. If there has been a sustained acceleration in the rate of CO_2 production, high initial CO_2 content and/or an unusually high rate of CO_2 production, the sample is determined positive (THORPE *et al.*, 1990).

We report the first 2 cases to our knowledge where the growth of *Plasmodium falciparum* triggered the BacT/Alert microbial detection system and played a role in making the diagnosis of malaria in the patients. In addition, further investigation using specimens obtained from one of the patients showed that the BacT/Alert culture medium is able to support the growth and maturation of *P. falciparum* for at least 72 h.

Case 1

A 35-year-old man who had arrived from Pakistan via Mozambique 1 week earlier presented to the Johannesburg Hospital with a 4-day history of malaise, fever, headache, vomiting and jaundice. On examination he had a temperature of 39°C, a pulse rate of 135/min and a blood pressure of 94/60 mmHg. He was confused and had terminal neck stiffness but no localizing signs. On auscultation his chest was clear and on examination of the abdomen no hepatosplenomegaly was noted. A generalized tonic–clonic seizure occurred in the ward. A working diagnosis of meningitis was made. A computerized tomography (CT) scan of the brain revealed no abnormality. A lumbar puncture was performed. Anaerobic and aerobic BacT/Alert culture bottles were each inoculated with approximately 10 mL of blood.

The full blood count taken on admission revealed a white cell count of $6{\cdot}3 \times 10^9$/L (normal range $3{\cdot}92$–$9{\cdot}88 \times 10^9$/L), a haemoglobin concentration of 13·8 g/dL (normal range 14·3–18·8 g/dL) and a platelet count of 3×10^9/L (normal range 140–400 $\times 10^9$/L). The haematocrit was 0·402 (normal range 0·43–0·55) and the red cell count was $4{\cdot}43 \times 10^{12}$/L (normal range $4{\cdot}89$–$6{\cdot}11 \times 10^{12}$/L). The lumbar puncture was com-

Table. Results of inoculation of BacT/Alert bottles with blood obtained from patients prior to initiation of antimalarial therapy

Patient	Bottle	Type	Volume of blood (mL)	Inoculum size (parasitized cells/L)[a]	Thin smear made from bottle contents after termination of incubation: Duration of incubation (h)	Thin smear made from bottle contents after termination of incubation: Appearance
2	A[b]	Aerobic	~8	$1{\cdot}24 \times 10^9$	23	Pyknotic, dysmorphic trophozoites
2	B[b]	Anaerobic	~8	$1{\cdot}24 \times 10^9$	48	Pyknotic, dysmorphic trophozoites
1	C[b]	Aerobic	~10	Unknown	108	Multiple infected cells, late trophozoites
1	D[b]	Anaerobic	~10	Unknown	12	Crenated red cells, late and disintegrated trophozoites
1	E	Anaerobic	5	$1{\cdot}6 \times 10^{12}$	72	Occasional late trophozoites
1	F	Aerobic	3	$9{\cdot}3 \times 10^{11}$	72	Occasional late trophozoites
1	G	Anaerobic	1	$3{\cdot}1 \times 10^{11}$	72	Occasional late trophozoites

[a]Calculated on basis of red cell count and % parasitaemia of specimen used to inoculate bottles.
[b]Original BacT/Alert blood cultures taken on admission.