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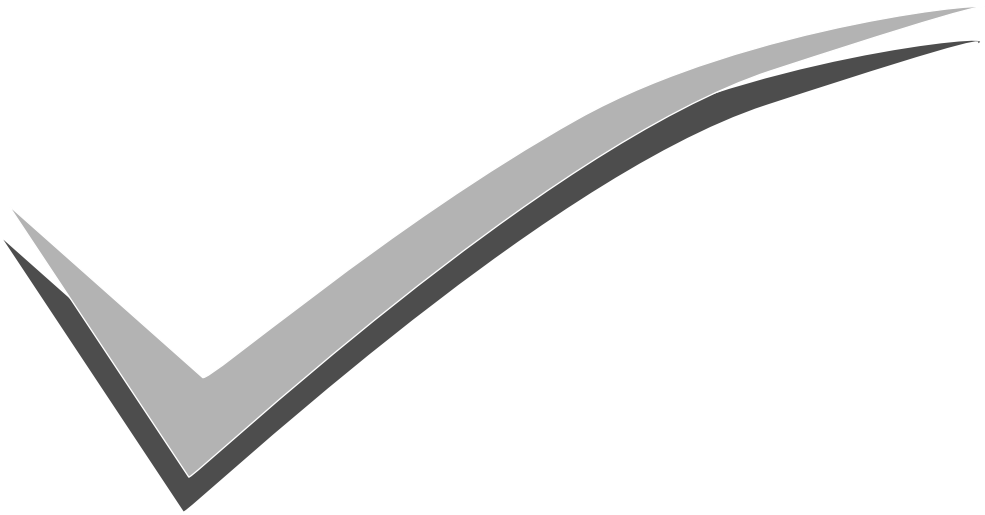

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OTHER EVALUATIONS

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S. No.	Name of the Evaluation Body
8.	National Institute of Malaria Research (ICMR), Goa, India
9.	Steel Authority of India, Bokaro Steel Plant, Bihar, India
10.	Apollo Hospital, Hyderabad, India

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Assessment of three new parasite lactate dehydrogenase (pan-pLDH) tests for diagnosis of uncomplicated malaria

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KEYWORDS
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Rapid diagnostic tests;
Validity;
Sensitivity and specificity;
Parasite lactate dehydrogenase tests

Summary A study to assess the diagnostic capabilities of three parasite lactate dehydrogenase (pan-pLDH) tests, Vistapan®, Carestart™ and Parabank®, was conducted in Uganda. An HRP2 test, Paracheck-Pf®, and a Giemsa-stained blood film were performed with the pLDH tests for outpatients with suspected malaria. In total, 460 subjects were recruited: 248 with positive blood films and 212 with negative blood films. *Plasmodium falciparum* was present in 95% of infections. Sensitivity above 90% was shown by two pLDH tests, Carestart (95.6%) and Vistapan (91.9%), and specificity above 90% by Parabank (94.3%) and Carestart (91.5%). Sensitivity decreased with low parasitaemia (χ^2 trend, $P < 0.001$); however, all tests achieved sensitivity >90% with parasitaemia $\geq 100/\mu l$. All tests had good inter-reader reliability ($\kappa > 0.95$). Two weeks after diagnosis, 4—10% of pLDH tests were still positive compared with 69.7% of the HRP2 tests. All tests had similar ease of use. In conclusion, two pLDH tests performed well in diagnosing *P. falciparum* malaria, and all pLDH tests became negative after treatment more quickly than the HRP2. Therefore the rapid test of choice for use with artemisinin-combination therapies in this area would be one of these new pLDH tests.

1. Introduction

The current gold standard for laboratory confirmation of malaria diagnosis is a peripheral blood film, examined microscopically. However, trained staff, quality equipment and supervision are often scarce within malaria-endemic

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populations, particularly in sub-Saharan Africa. Clinicians often have to rely on clinical signs and symptoms for diagnosis, and, where there is also an increasing emphasis on home-based management, malaria diagnosis is often equated with fever (Ministry of Health, Uganda, 2003). Such presumptive treatment without laboratory confirmation could contribute to the development of drug resistance (WHO, 2000a).

An alternative diagnostic method to the blood film is the rapid diagnostic test (RDT), recommended by WHO where reliable microscopy is not available (WHO, 2000a). RDTs are antigen detection tests, which are simple to use and interpret and also use peripheral blood. The most commonly used RDT detects histidine-rich protein II (HRP2), produced by trophozoites and young gametocytes of *Plasmodium falciparum*. HRP2 tests have been the most widely evaluated to date and show consistently high sensitivity (Gaye et al., 1999; Guthmann et al., 2002; Van den Ende et al., 1998; Wolday et al., 2001). However, they are limited in that they detect *P. falciparum* only and can remain positive for several weeks after antimalarial treatment (Beadle et al., 1994; Humar et al., 1997). Both these factors can exacerbate drug resistance: for example, due to suspicion of non-falciparum malaria with a negative test, and false positive tests occurring in recently treated individuals presenting to a clinic with alternative pathologies.

A second type of RDT detects the malaria antigen parasite lactate dehydrogenase (pLDH), an enzyme produced in the glycolytic cycle of the asexual stage of all species of *Plasmodium*. pLDH is produced only by viable parasites, thus being cleared from the bloodstream more quickly after treatment, resulting in the test becoming negative more quickly (Laferi et al., 1997; Piper et al., 1999; Wu et al., 2002). These characteristics suggest that pLDH tests could be used with more confidence for malaria diagnosis at the peripheral level. However, when previously available pLDH tests have been evaluated they have shown much variability in sensitivity, ranging from 60.4% (Fryauff et al., 2000) to 100% (Pattanasin et al., 2003).

The development of several new pLDH monoclonal antibodies by Flow Inc. (Portland, OR, USA) has enabled the production of a new generation of pLDH tests. This paper reports the results of a field evaluation of three of these tests, namely Vistapan® malaria test (Mitra, New Delhi, India), the Carestart™ antigen test (AccessBio, Princeton, NJ, USA) and the Parabank® device (Orchid/Zephyr, Goa, India), to assess their validity, inter-reader reliability, ease of use and persistence of positive tests following efficacious treatment. An HRP2 test, Paracheck-Pf® (Orchid/Zephyr, Goa, India), previously shown to be the most appropriate HRP2 test for field use in this setting (Guthmann et al., 2002), was also included in the study.

2. Materials and methods

2.1. Study site

The study was conducted in Mbarara Regional Referral Hospital, situated in a mesoendemic area of malaria transmission in southwestern Uganda.

2.2. Enrolment of study patients

Patients from the outpatient department were systematically screened for symptoms suspected to be malaria and referred to the research clinic. Inclusion criteria were a clinical suspicion of malaria; weight $\geq$5 kg; resident in Mbarara Municipality; available for a 2 week follow-up period; and signed informed consent from the study subjects or their legal guardians. Exclusion criteria were signs of severe or complicated malaria (WHO, 2000b); signs of severe disease; and women with visible pregnancy or suspicion of pregnancy based on an assessment of the last normal menstrual period. Ineligible or non-consenting patients were managed appropriately.

2.3. Sample size

The required number of patients with a positive blood film was calculated using an estimated sensitivity of the RDTs of 90%, an alpha error of 0.05 and a precision of 6%. This number (n = 96) was doubled to permit a stratified analysis by age group (0—4 and $\geq$5 years). The same parameters were used to calculate the required number of patients with a negative blood film, thus giving a final minimum sample size of 200 blood-film-positive and 200 blood-film-negative patients.

2.4. Study procedures

On the day of inclusion, demographic and clinical information was recorded, and a thick/thin blood film and the four rapid tests (Vistapan, Carestart, Parabank and Paracheck-Pf) were performed. Women with a positive pregnancy test and hyperparasitaemic patients (*P. falciparum* >250 000 parasites/μl) were given quinine and excluded from further follow-up. All other patients with a positive blood film received an artemether—lumefantrine (Coartem®, Novartis Pharma AG, Basel, Switzerland) six-dose regimen under directly observed therapy. This regimen has been shown to be highly efficacious (Piola et al., 2005), with a prompt reversion to a negative blood film after treatment. Patients receiving Coartem were asked to return to the clinic on the third, seventh and 14th day after inclusion to repeat the blood film and all RDTs.

2.5. Laboratory procedures

Blood films and rapid tests were performed from the same fingerprick blood. Blood films were dried, thin films fixed in methanol, and both films stained with 3% Giemsa for 45 min. Smears were read by experienced technicians, counting parasites against 200 or 500 white blood cells (WBC) or 200 high power fields before declaring a blood slide negative. The parasite density per microlitre was calculated by multiplying the asexual parasite count by 8000 and dividing by the number of WBC counted (WHO, 1991). *Plasmodium* species were confirmed on the thin film and slides with mixed infections had only *P. falciparum* quantified. Slides with a non-falciparum monoinfection had the asexual density per microlitre calculated as for *P. falciparum*. Gametocytes were recorded with species identification where possible,

with density counts for *P. falciparum* only. All inclusion slides were blinded and double-read, with a third reading performed in case of discordance, i.e.: positive/negative discordance for asexual stages; species discordance for asexual stages; asexual density discordance (difference in parasitaemia ≥50%); positive/negative gametocyte discordance. Twenty percent of follow-up visit slides were also blinded and double-read. External quality control of 290 inclusion slides was performed by Shoklo Malaria Research Unit, Thailand, giving Mbarara laboratory a sensitivity of 95.5% and a specificity of 100%.

All RDTs were performed and interpreted according to the manufacturers' instructions. A loop or pipette was used to transfer blood from the finger onto the test. Buffer solution was applied, and this carried the blood up the cellulose nitrate strip, over the test and control lines. pLDH present in positive samples bound with the colloidal gold anti-pLDH antibody and was captured by the anti-pLDH test line on the test strip to produce a visible line. Results were read at either 15 or 20 min (according to the test). The presence of a control and test line denoted a positive test, while a control line only denoted a negative test. Absence of a control line indicated an invalid test, which was then repeated.

Each test result was interpreted by two independent health care providers blind to the result of the blood film and reading according to a rota to avoid observer bias. The first reading was performed at the time specified by the manufacturer (15 min after preparation for Paracheck-Pf and Parabank and 20 min for Carestart and Vistapan). The second reading was performed within 15 min of the first one. Discordant results were read by the laboratory supervisor for a definitive result. Each reader also classified the test as either invalid or doubtful. A doubtful test was defined as a test for which the reader was not sure if there was any indication of a line present.

At the end of the study, two test readers and two laboratory technicians involved in preparing the tests completed a questionnaire concerning the ease of use and interpretation of each test.

2.6. Outcomes

The main study outcome was the validity of the RDTs on the day of diagnosis: i.e. the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Sensitivity was defined as the percentage of positive tests among the total number of positive blood slides. Specificity was defined as the percentage of negative tests among the total number of negative blood slides. The PPV was defined as the percentage of positive blood slides among the total number of positive tests. The NPV was defined as the percentage of negative blood slides among the total number of negative tests. The three other outcomes were: (1) inter-reader reliability, i.e. the extent to which the interpretation of the test differed between two readers; (2) percentage of tests remaining positive on follow-up; and (3) 'ease of use', assessed by a questionnaire with five sections (ease of performance, safety, stability of the result, interpretation and storage). Each section was weighted according to its perceived importance, and a total score (out of 100) was derived from the sum of the weighted sections.

2.7. Analysis

All data were either recorded directly or transcribed from source data forms to an individually numbered case report form (CRF). Data were double-entered and validated using EpiData version 3.1 (EpiData Association, Odense, Denmark) and analysed using Stata 9.1 (Stata Corp., College Station, TX, USA). The study profile and baseline characteristics were summarised, including comparative tests between age groups (χ^2 test, Mann-Whitney *U* test). Validity for each test was calculated overall and then stratified by age group, level of parasitaemia (parasites/μl 1–99, ≥100, ≥200, ≥500), presence/absence of fever, duration of illness (0–2 vs. 3 d and above) and a history of taking antimalarials, using comparative tests (χ^2 test, Mann-Whitney *U* test) to compare differences between groups. Kappa statistics were calculated for inter-reader reliability for each test on the day of diagnosis. A test was considered reliable if $\kappa \geq 0.8$. Univariate and multivariate analyses were performed to investigate the association between explanatory factors and the test remaining positive at each follow-up visit.

3. Results

3.1. Demographic and parasitological characteristics of study subjects

Between 26 April and 27 July 2005, 485 patients from the outpatient department were screened. Nine were ineligible (three had severe illness, five were non-residents and one was not in the appropriate age group after completion of recruitment in the under fives). Sixteen patients did not consent to participate in the study. Therefore, 460 patients were included in the study: 239 under fives and 221 aged 5 years and above. The mean age was 12 years (SD 13 years; Table 1). There were 248 positive blood films with *P. falciparum* monoinfections (93.6%), *P. malariae* monoinfections (2.4%), *P. vivax* monoinfections (2.4%), *P. falciparum* + *P. malariae* mixed infections (0.8%) and *P. falciparum* + *P. vivax* mixed infections (0.8%). Of the 212 negative films, nine had gametocytes present. Parasitological characteristics of positive subjects are given in Table 1. Slides positive with *P. falciparum* had higher parasite densities than those of the other two species.

3.2. Validity of RDTs

Only Carestart had estimates for all validity parameters greater than 90% (Table 2). Vistapan and Carestart were as sensitive as Paracheck-Pf ($P = 0.14$ and $P = 0.38$, respectively). Parabank was less sensitive than all other tests ($P < 0.001$ for each comparison). There was no significant difference in specificity between the three pLDH tests, but Parabank had a higher specificity compared with Paracheck-Pf ($P = 0.02$) for *P. falciparum* detection. Sensitivity decreased with older age for both Vistapan [97.7% (under fives) vs. 85.7%, $P < 0.01$] and Parabank [95.4% (under fives) vs. 73.1%, $P < 0.001$]. Sensitivity increased with axillary temperature ≥37.5 °C at inclusion for Paracheck-Pf (98.8 vs. 91.4%, $P = 0.04$), Vistapan (97.6 vs. 89.0%, $P = 0.03$) and

Table 1 Baseline characteristics of all study subjects and parasitological characteristics of slide-positive subjects attending Mbarara Regional Referral Hospital outpatient department, southwestern Uganda

	Group A (<5 years)	Group B (≥5 years)	Overall	*P*-value
Baseline characteristic	*n* = 239	*n* = 221	*n* = 460	
Gender ratio (M:F)	0.98 (118:121)	0.52 (76:145)	0.73 (194:266)	0.001 (χ^2)
Mean age (SD)	2 years (14 months)	22 years (12 years)	12 years (13 years)	N/A
Median duration of illness in days (range)	3 (1—14)	3 (1—30)	3 (1—30)	0.2 (Kruskal-Wallis)
Previously taken antimalarials (*n*, %)	81 (33.9)	60 (27.3)	141 (30.7)	0.13 (χ^2)
Fever on presentation (axillary temp. ≥37.5 °C)	99 (41.4)	31 (14.0)	130 (28.3)	<0.001 (χ^2)
Parasitological characteristic	*n* = 129	*n* = 119	*n* = 248	
Asexual parasitaemia range (parasites/μl)	16—703 411	16—233 241	16—703 411	0.001 (Kruskal-Wallis)
Geometric mean of asexual parasitaemia (95% CI)	7433 (4869—11 346)	1524 (975—2384)	3475 (2521—4790)	0.001 (*t* test)
Interquartile range (interquartile value)	1682—45 748 (44 066)	166—11 070 (10 904)	641—23 827 (23 186)	—
Gametocyte carriage (*n*, %)	36 (27.9)	22 (18.5)	58 (23.4)	0.11 (χ^2)

Parabank (91.8 vs. 81.0%, $P = 0.04$) compared with patients with axillary temperatures <37.5 °C. Differences in sensitivity according to age and baseline temperature were no longer present after stratification by level of parasitaemia (<100 vs. ≥100 parasites/μl). Sensitivity was above 90% for all tests in subjects with parasitaemias ≥100 parasites/μl, and significantly higher than for levels of parasitaemia <100/μl (Paracheck-Pf, 96.3 vs. 77.4%; Vistapan, 96.8 vs. 58.1%; Carestart, 99.5 vs. 67.7%; Parabank, 90.8 vs. 41.9%; $P < 0.001$ for all comparisons). No significant differences in sensitivity were found between patients presenting before or after 2 d of onset of the episode, or according to a history of taking antimalarials in the previous 14 d.

Although the small number of non-falciparum monoinfections does not permit reliable calculation of validity of non-falciparum malaria, all tests detected 100% ($n = 6$) of the *P. malariae* monoinfections. *Plasmodium vivax* was detected in 4/6 infections by Carestart, 2/6 by Vistapan and 1/6 by Parabank.

3.3. Reliability

The κ statistic for the inter-reader reliability for all tests was above 0.90 (very good agreement) [Carestart, $\kappa = 0.96$ (95% CI 0.94—0.99); Vistapan, $\kappa = 0.94$ (95% CI 0.91—0.97); Parabank, $\kappa = 0.96$ (95% CI 0.94—0.99); Paracheck-Pf, $\kappa = 0.97$ (95% CI 0.95—1.0)].

3.4. Time to negativity of RDTs

There were no positive blood films on follow-up visits, and therefore every positive RDT result on day 3, 7 or 14 was considered a false positive result (Table 3). All three pLDH tests had significantly fewer false positive tests on every day of follow-up compared with Paracheck-Pf ($P < 0.001$ for all tests on days 3, 7 and 14). There was no difference between the pLDH tests by day 14, with the percentage of positive tests ranging from 4.6 to 9.5%.

Younger age group and higher parasite level at inclusion were related to positive Paracheck-Pf on all follow-up days (logistic regression, $P < 0.01$) for all. Age group, fever at diagnosis and presence of gametocytes on day 3 were all related to a positive pLDH test on day 3 (except age group for Parabank) (age group: Vistapan $P = 0.026$, Carestart $P < 0.001$; fever on day 0: Vistapan $P = 0.001$, Carestart $P < 0.001$, Parabank $P = 0.01$; gametocytes $P < 0.001$ for all tests). No overall associations could be made for days 7 and 14, but factors such as fever at day 0, presence of gametocytes and parasite density at day 0 were implicated (data not shown due to small numbers).

Table 2 Validity of four rapid diagnostic tests for the detection of *Plasmodium* species in patients attending Mbarara Regional Referral Hospital outpatient department, southwestern Uganda

	Carestart % [95% CI]	Vistapan % [95% CI]	Parabank % [95% CI]	Paracheck-Pf % [95% CI]
Sensitivity	95.6 (237/248) [90.2—96.6]	91.9 (228/248) [87.8—95]	84.7 (210/248) [79.6—88.9]	94 (233/248) [90.2—96.6]
Specificity	91.5 (194/212) [86.9—94.9]	89.6 (190/212) [84.7—93.4]	94.3 (200/212) [90.3—97.0]	87.3 (185/212) [82.0—91.4]
PPV	92.9 (237/255) [89.1—95.8]	91.2 (228/250) [87—94.4]	94.6 (210/222) [90.7—97.2]	89.6 (233/260) [85.3—93]
NPV	94.6 (194/205) [90.6—97.3]	90.5 (190/210) [85.7—94.1]	84.0 (200/238) [78.7—88.4]	92.5 (185/200) [87.9—95.7]

PPV: positive predictive value; NPV: negative predictive value.

Table 3 Percentage of positive tests on each follow-up visit in patients attending Mbarara Regional Referral Hospital outpatient department, southwestern Uganda

	Day 0 *n*[a]	Day 3 % [95% CI]	Day 7 % [95% CI]	Day 14 % [95% CI]
Paracheck-Pf	226	86.2 (193/224) [81.7—90.7]	80.8 (181/224) [75.6—86.0]	69.7 (152/218) [63.1—75.7]
Vistapan	221	36.1 (79/219) [29.7—42.5]	23.4 (51/218) [17.8—29.0]	8.9 (19/213) [5.1—12.7]
Carestart	230	42.5 (97/228) [36.1—48.9]	27.6 (63/228) [21.8—33.4]	9.5 (21/221) [5.6—13.4]
Parabank	204	17.8 (36/202) [12.5—23.1]	8.9 (18/202) [5.0—12.8]	4.6 (9/196) [1.7—7.5]

[a] *n* is the number of positive tests for each RDT on day 0 in patients who were followed up.

3.5. Ease of use

Overall, there were no large differences between the tests in terms of ease of use. Some tests had small advantages or disadvantages: for example, Vistapan had individual buffer sachets, considered to be an advantage, whereas Carestart had a delay of 60 s between the blood application and the buffer application, considered to be a disadvantage. The differences in structure of the blood collection device, either incomplete loops, a full loop or a micropipette, led to differences in perceived safety (loops were considered to have a risk of splashing the blood into the eyes of the technician) and ease of filling and emptying the device. There were more doubtful tests on follow-up visits, particularly for Carestart and Vistapan, as the positive test line became progressively fainter. All test results were stable for a minimum of 24 h. The number of invalid tests was <0.5% for Parabank and between 0.5 and 2% for Carestart and Vistapan. No test had items requiring refrigeration and all tests have undergone temperature stability studies up to 30 °C.

4. Discussion

This is the first study to evaluate a new generation of pLDH tests for malaria diagnosis, performed in a mesoendemic African setting with a predominance of *P. falciparum* infections. We showed that several of these tests proved valid, reliable and easy to use, and should be of great use in malaria-endemic countries where microscopy not available, particularly in emergency settings.

For confident diagnosis of malaria in routine outpatient department conditions, a sensitivity of more than 90% is crucial, and this was achieved by both Carestart and Vistapan. The pLDH tests also demonstrated desirable qualities that could reduce the possibility of patients without malaria being given antimalarials and therefore could reduce drug pressure, a major concern at a time when artemisinin combination therapies (ACTs) are being introduced throughout Africa. Firstly, their high specificity would reduce the number of patients with a false positive test being treated for malaria. Secondly, the great reduction in the number of tests remaining positive after treatment compared with the HRP2 test would reduce the number of false positive tests in febrile patients returning to the clinic shortly after efficacious antimalarial treatment. Thirdly, the ability to detect both *P. malariae* and *P. vivax* would increase confidence in a negative test, although in the study population these species are infrequent and the mean parasite density for *P. vivax* was low, which could have contributed towards the relatively poor detection of this species. The excellent inter-reader reliability of all the tests when interpreted by a variety of non-laboratory staff and their simple utilisation would enable any health staff to be trained to use and interpret the tests accurately. This is an advantage in countries where trained laboratory staff are scarce and cadres such as nursing assistants are frequently in the front line for providing clinical care and diagnosis for patients in health outposts.

A variety of factors may contribute towards the differing sensitivity of the test, such as patient age and parasitaemia, which will vary according to endemicity. Lower test sensitivity related to low parasitaemia in adults in an area of stable transmission is a limitation of the tests. Although such patients are less at risk from severe clinical episodes, they perpetuate parasite transmission, and are still a public health concern. The new pLDH tests should be tested in a variety of epidemiological situations to assess their local performance, especially in places where *P. vivax* monoinfections are more prevalent, such as Asia and South America.

The faster clearance of pLDH after efficacious treatment indicates that pLDH tests could be useful in monitoring treatment efficacy, although results within the first 2 weeks would still need to be treated with caution, as gametocytes in the circulation on or after day 3 could indicate a false positive pLDH test.

The frequency of doubtful or invalid tests was at a reasonable level for operational use. Continuing real-time temperature stability studies up to 50 °C are necessary to ensure test viability at temperatures such as those that may be attained in the field, where ideal storage conditions are difficult to maintain. The current price of pLDH tests is between US$0.60 and US$1.00: between 15 and 55 cents more than the HRP2 test (US$0.45/test). If these tests are to be affordable in public health programmes, their cost must be reduced to below US$0.50/test. The current move to introduce ACTs into sub-Saharan Africa with the financial support provided by the Global Fund needs to be in line with confirmed diagnosis to reduce antimalarial prescriptions on clinical grounds only, and to rationalise health budgets in view of the much higher costs of the ACTs. If pLDH tests were more affordable, it would be more feasible for health outposts, currently relying on clinical diagnosis, to incorporate RDTs into their diagnostic algorithms. The reduced expenditure on ACTs for negative patients could balance the extra costs of using RDTs (Rolland et al., 2006). A basic cost comparison of malaria diagnosed clinically versus confirmed diagnosis using rapid tests based on figures at the study site demonstrated a cost saving of 29% using RDTs (see Appendix), due to the reduction in overdiagnosis and there-

fore overtreatment with ACTs. Such analysis could be done by individual health ministries to assess the feasibility of introducing RDTs on a wide scale.

In conclusion, after confirmatory testing in a variety of epidemiological situations, this new generation of pLDH rapid diagnostic tests should be a useful adjunct in the fight against malaria. Used in conjunction with ACTs, they could reduce the risk of drug resistance.

Author's contributions

All authors contributed to the study protocol, coordinated by CF and JPG; CF, RT, VB, PP and CN supervised the overall implementation of the study; JK and FM supervised the laboratory activities; CF performed the analyses and drafted the manuscript. All authors read and approved the final manuscript. CF and JPG are guarantors of the paper.

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Conflicts of interest: None declared.

Ethical approval: The Faculty of Medicine Research and Ethics Committee, the Institutional Review Board of Mbarara University of Science and Technology, Uganda and the Uganda National Council for Science and Technology (national ethics committee).

Appendix A

A simple model was created to assess the net cost effect of including RDTs in the algorithm for malaria diagnosis where treatment with an ACT is used. In 2003, 75% of <5 consultations ($n = 11\,200$) and 20% of >5 consultations ($n = 59\,000$) in Mbarara outpatient department were attributed to malaria. Assuming all suspected malaria cases were treated (with Coartem costing US$1.2 for children and US$2.0 for adults) and a blood film was taken for 10% of attending patients (at a cost of US$0.3 including human resource costs), the total cost of malaria diagnostics and treatment (not including clinicians' salaries) could be estimated at US$38 648.

If RDTs were available, more tests may be used than the number of currently suspected malaria cases. Therefore, using the same consultation figures and assuming that 80% of children <5 years and 35% of >5 years had an RDT performed, with two laboratory technicians to process the tests, and assuming (based on Epicentre observations of the proportion of an age group with a positive blood film) 50% positive RDTs in the <5 years group and 30% positive RDTs in the >5 years group, the cost of Coartem and extra laboratory human resources plus the RDTs would be US$27 309.

This represents a saving, for this hospital alone, in 1 year, of US$11 339 (ca. 30%), assuming that only RDT positive cases are treated.

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MALARIA JOURNAL

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Health facility-based malaria surveillance: The effects of age, area of residence and diagnostics on test positivity rates

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Abstract

Background: The malaria test positivity rate (TPR) is increasingly used as an indicator of malaria morbidity because TPR is based on laboratory-confirmed cases and is simple to incorporate into existing surveillance systems. However, temporal trends in TPR may reflect changes in factors associated with malaria rather than true changes in malaria morbidity. This study examines the effects of age, area of residence and diagnostic test on TPR at two health facilities in regions of Uganda with differing malaria endemicity.

Methods: The analysis included data from diagnostic blood smears performed at health facilities in Walukuba and Aduku between January 2009 and December 2010. The associations between age and time and between age and TPR were evaluated independently to determine the potential for age to confound temporal trends in TPR. Subsequently, differences between observed TPR and TPR adjusted for age were compared to determine if confounding was present. A similar analysis was performed for area of residence. Temporal trends in observed TPR were compared to trends in TPR expected using rapid diagnostic tests, which were modelled based upon sensitivity and specificity in prior studies.

Results: Age was independently associated with both TPR and time at both sites. At Aduku, age-adjusted TPR increased relative to observed TPR due to the association between younger age and TPR and the gradual increase in age distribution. At Walukuba, there were no clear differences between observed and age-adjusted TPR. Area of residence was independently associated with both TPR and time at both sites, though there were no clear differences in temporal trends in area of residence-adjusted TPR and observed TPR at either site. Expected TPR with pLDH- and HRP-2-based rapid diagnostic tests (RDTs) was higher than observed TPR at all time points at both sites.

Conclusions: Adjusting for potential confounders such as age and area of residence can ensure that temporal trends in TPR due to confounding are not mistakenly ascribed to true changes in malaria morbidity. The potentially large effect of diagnostic test on TPR can be accounted for by calculating and adjusting for the sensitivity and specificity of the test used.

Background

As malaria control efforts intensify, there is a vital need to accurately measure changes in the burden of disease and to evaluate the impact of control interventions through improved surveillance [1]. Malaria incidence, defined as the number of malaria cases per person-time, is a core indicator of the burden of disease and endorsed by the World Health Organization (WHO) [2]. For countries in sub-Saharan Africa where malaria morbidity is highest, malaria incidence is typically estimated based on the number of reported cases of malaria captured through the health management information system (HMIS) per population at risk. Incomplete reporting and lack of laboratory confirmation limit the accuracy of these data [2]. To account for those limitations, many countries also report the incidence of laboratory-confirmed cases, but these data may reflect the availability and utilization of clinical and laboratory services rather than malaria morbidity in the population [2]. Many studies of malaria control interventions such as indoor residual spraying or

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distribution of insecticide-treated nets use outcomes that are simpler to measure than malaria incidence, such as number of episodes of uncomplicated malaria (without a denominator), asymptomatic parasitaemia prevalence, haemoglobin levels, or all-cause child mortality [3,4].

The malaria test positivity rate (TPR), defined as the proportion of diagnostic tests that are positive for malaria, is an alternate indicator of malaria morbidity [2]. TPR is similar to the slide positivity rate (SPR) except that it includes the results of rapid diagnostic tests (RDTs) when they are used in addition to or in place of blood smears. TPR has been used as a surveillance indicator at the national [2] and regional level [5], and decreases in TPR have been used as evidence to support the effectiveness of malaria control interventions [6-8]. The advantages of TPR are that it inherently incorporates only laboratory-confirmed cases, provides a clear denominator and can provide a rapid and inexpensive means of assessing malaria morbidity in a population utilizing health care facilities where diagnostic testing is available. As it has been previously reported, a disadvantage of TPR is that differences over time or across populations may reflect differences in the incidence of non-malarial febrile illnesses rather than differences in the burden of malaria [9]. In addition, temporal trends in TPR may reflect changes in other factors associated with malaria diagnosis, such as the age or area of residence, the proportion and selection of individuals tested or the sensitivity and specificity of the diagnostic test used.

As the WHO now recommends laboratory confirmation for all patients suspected of having malaria before treating [10], the TPR has become an increasingly practical indicator of malaria morbidity. In this study, data from a health facility-based malaria surveillance system at two sites in Uganda with differing epidemiology were used to characterize the effects of age and area of residence on temporal trends in TPR. Temporal trends in TPR were also modelled using different diagnostic tests, including microscopy and RDTs, which would be expected affect TPR due to differences in sensitivity and specificity compared to microscopy.

Methods

Description of study sites and data collection

The Uganda Malaria Surveillance Project (UMSP) in collaboration with the Uganda National Malaria Control Programme (NMCP) established a health facility-based malaria surveillance system at six sentinel sites between September 2006 and January 2007. Detailed descriptions of study sites and data collection have been published previously [11]. Briefly, all sentinel site facilities are level IV government run health centres with a catchment population of approximately 100,000 people. They provide care free of charge, including diagnostic testing and medications. The two sites selected for this study, Aduku and Walukuba, represent contrasting malaria transmission settings in Uganda with previously reported entomological inoculation rates (infective mosquito bites per person per year) of 1,564 and 6, respectively [12]. This analysis included data collected between January 2009 and December 2010 at two of the six sentinel sites. Individual-level patient data collected for all patients presenting to the outpatient clinics of the health facilities included age, village and parish of residence, whether a blood smear was performed, presence or absence of asexual parasites based on a thick blood smear, in addition to other demographic information, basic clinical information, laboratory results, diagnoses, and treatments prescribed. Data were entered electronically using Epi Info version 3.5.1 (Centers for Disease Control and Prevention, Atlanta, GA, USA) at the sites and sent once a month to a core facility in Kampala for uploading to a SQL server (Microsoft Corporation, Redmond, WA, USA). A public website exists where standardized tables and figures can be generated to monitor trends in key indicators and monthly reports are posted [13].

Definition of variables

Age data were collected as months and years up to age 5, and as years only after age five. Age was classified as less than 5, 5 to 15, and greater than 15 years in analyses of the relationships between age and TPR and between age and calendar time. In adjusting for age, visits at each site were separately categorized into 20 equivalent age quantiles, each containing 5% of visits at that site. Area of residence categories were determined based upon the reported parish of residence. Parishes that contributed to $< 1\%$ of cases that underwent diagnostic testing were combined into an "other" category. There were 24 categories of calendar time based upon the month and year. Suspected malaria was defined as all patients referred for malaria laboratory testing plus all patients not referred for a malaria laboratory test, but given a clinical diagnosis of malaria. TPR was defined as the proportion of tests (all of which were blood smears) positive for malaria.

Expected TPR (TPR_{exp}) for an RDT was calculated from the observed TPR (TPR_{obs}) as follows:

$$TPR_{\exp} = (TPR_{obs}\ x\ sensitivity) + (1 - TPR_{obs})\ x\ (1 - specificity)$$

The sensitivity and specificity of RDTs was determined during a prior study at these sites conducted between May 2006 and February 2007 [14]. For histidine rich protein-2 (HRP-2) based RDTs (Paracheck; Orchid Biomedical Systems) sensitivity and specificity were 99.7% and 38.1% at Aduku, and 97.4% and 69.6% at Walukuba,

respectively. Sensitivity and specificity of Plasmodium lactate dehydrogenase (pLDH) based RDTs (Parabank; Zephyr Biomedicals) were 98.6% and 69.9% at Aduku, and 92.3% and 81.9% at Walukuba, respectively.

Statistical analysis

The potential for confounding by age and/or area of residence in the association between the exposure of interest, calendar time, and the outcome of interest, TPR, was investigated. Analyses of the relationships between age and area of residence and temporal trends in TPR included only those patients for whom a thick blood smear was performed.

To investigate the potential for age to confound the relationship between calendar time and TPR, the associations between age and calendar time and between age and TPR were evaluated separately using the Pearson Chi-square test. To visually inspect the degree to which confounding by age occurred, temporal trends in TPR were adjusted using direct standardization based on the distribution of visits among 20 age categories of equal size over the entire time period, and then compared to unadjusted temporal trends in TPR.

To investigate the potential for area of residence to confound the relationship between calendar time and TPR, the associations between area of residence and calendar time and between area of residence and TPR were also separately evaluated using the Pearson Chi-square test. To visually inspect the degree to which confounding by area of residence occurred, temporal trends in TPR were similarly adjusted for area of residence using direct standardization based on the distribution among parishes over the entire study period, and then compared to unadjusted temporal trends in TPR.

To characterize the effect of diagnostic test on temporal trends in TPR, an expected value for TPR was calculated using RDTs based on the sensitivity and specificity of those tests from a previous study from the same two sentinel sites using quality-controlled microscopy as a gold standard [14], as described above. All analyses were performed using R, version 2.9.1. P values <0.05 were considered statistically significant.

Results

Characteristics of visits

The characteristics of outpatient visits at the two sites are summarized in Table 1. More than 96% of visits had data on age and area of residence. The proportion of patients suspected of having malaria ranged from 53-55 % at the two sites. At both sites, 95% of those with suspected malaria received a thick blood smear. A higher proportion of those receiving a thick blood smear were under five at Aduku compared to Walukuba (44% *vs* 29%, $p < 0.001$). Overall TPR for the entire study period was higher at Aduku compared to Walukuba (53% *vs* 40%, $p < 0.001$).

Table 1 Characteristics of outpatient visits at surveillance sites in 2009-10

Characteristics	Surveillance Site	
	Walukuba	Aduku
Visits with complete data* (% of total visits)	71,703 (98%)	38,912 (96%)
Number with suspected malaria (% with complete data)	37,806 (53%)	21,570 (55%)
Number with blood smear (% of suspected)	36,079 (95%)	20,488 (95%)
Under 5 (% of blood smears)	10,636 (29%)	9,052 (44%)
5 to 15 (% of blood smears)	8,755 (24%)	2,644 (13%)
Over 15 (% of blood smears)	16,688 (46%)	8,752 (43%)
Number with positive blood smear (TPR)	14,391 (40%)	10,806 (53%)

* Includes age and area of residence (parish).

The effect of age on temporal trends in malaria test positivity rate

Changes in TPR over time could be confounded by age, which has a well known association with malaria infection and morbidity. As expected, TPR varied significantly by age group in Aduku. For visits by patients under five years of age, five to 15 and over 15, TPR was 71%, 64%, and 30%, respectively ($p < 0.001$). In Walukuba, the association between TPR and age was less dramatic (44%, 47%, and 34% for under five, five to 15 and over 15, respectively), but remained statistically significant ($p < 0.001$).

For age to confound temporal trends in malaria TPR, it must also be associated with calendar time, which was the case at both sites in this study. In Aduku, the proportion of visits by patients under age five varied from a high of 56% in September 2009 to a low of 24% in December 2010 ($p < 0.001$). There was an upward trend in age at Aduku throughout the study period with the proportion of visits by patients under age five ranging between 44% and 55% in the first six months of the study period, and between 24% and 40% in the final six months. Walukuba demonstrated less variation in the distribution of age over time, and did not exhibit any consistent trend, but differences remained statistically significant. The proportion of patients under age five varied from a high of 41% in April 2009 to a low of 23% in June 2010 ($p < 0.001$).

Given the significant associations between both age and calendar time, and age and TPR, the potential for confounding was present at both sites. To determine the degree to which confounding actually occurred, temporal trends in the observed TPR and TPR adjusted for age were compared as shown in Figure 1. Subtle but clear confounding by age was observed in Aduku, where the difference in TPR adjusted for age compared to the

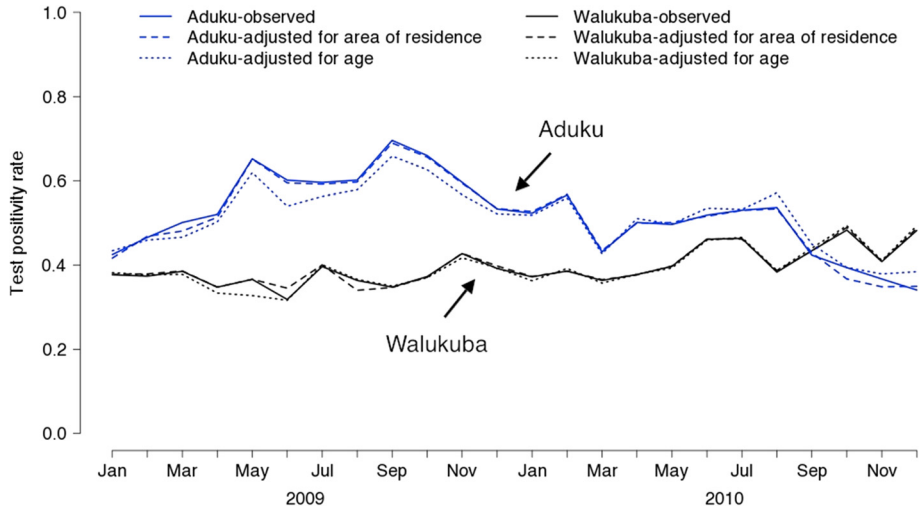

Figure 1 TPR-observed, TPR-adjusted for age, and TPR-adjusted for area of residence at Walukuba and Aduku health centers (TPR = test positivity rate).

observed TPR gradually increased over calendar time. This bias could be predicted based upon the gradual increase in age, and the strong association between young age and a positive thick blood smear. As a result of confounding by age, the decline in the observed TPR from 69% in September 2009 to 34% in December 2010, was greater than the decline in TPR adjusted for age from 66% to 38% over the same time period. In contrast, Walukuba demonstrates minimal evidence of confounding by age as the temporal trends in observed TPR and TPR adjusted for age are nearly identical over the entire study period. The largest difference in the monthly trend occurs between May and June of 2009 when the observed TPR declined 5% and the TPR adjusted for age declined 1%.

The effect of area of residence on trends in malaria test positivity rate

The association between area of residence and TPR is also well known, and was of interest in this study. Visits were categorized based on the parish where the patient lived, and visits from parishes contributing fewer than 1% of the cases for the entire study period were grouped into a category labelled "Other". This created 8 regions surrounding Walukuba and 18 surrounding Aduku. Table 2 shows the frequency distribution of the area of residence categories, average distance from the parish to the health facility and TPR over the entire study period in the regions surrounding Walukuba and Aduku. In Walukuba, TPR varied significantly across the areas of residence ($p < 0.001$), ranging from 38% to 53%. In Aduku, TPR varied significantly across the areas of residence ($p < 0.001$), ranging from 41% to 60%. There was no clear pattern between the distance from the area of residence to the clinic and the TPR at either site.

Although there were significant associations between area of residence and TPR at both sites, the distribution of area of residence among patients undergoing diagnostic testing would need to vary significantly over time for the potential for confounding to be present, and this was the case at both sites. For example, the proportion of patients who underwent diagnostic testing who were from the Masese Parish ranged from 30% in October 2009 to 39% in December 2010 at Walukuba and patients who were from the Ongoceng Parish ranged from 17% in August 2009 to 25% in October 2009 at Aduku ($p < 0.001$ in both cases).

Given the associations with both TPR and calendar time, area of residence was a potential confounder. As was done for age, temporal trends in observed TPR and TPR adjusted for area of residence were compared, however, they differed only slightly and without any clear pattern (Figure 1). The largest difference in monthly trends at Aduku occurred between November 2010 and December 2010 when observed TPR declined 3% and TPR adjusted for area of residence was unchanged. In Walukuba, the largest difference in monthly trends occurred between May and June of 2009 when observed TPR decreased 5% and TPR adjusted for area of residence decreased 2%.

The effect of diagnostic test on trends in malaria test positivity rate

Figure 2 shows trends in observed TPR using microscopy compared to those expected using RDTs based on HRP-2 and pLDH. At both sites, expected TPR with pLDH- and HRP-2-based RDTs is higher than observed TPR. The differences are greater at Aduku than at Walukuba, and greater with HRP-2-based RDTs than with

Table 2 Distribution of area of residence and TPR

Surveillance Site							
Walukuba				Aduku			
Parish	**Distance***	**Frequency**	**TPR**	**Parish**	**Distance***	**Frequency**	**TPR**
Masese	3.8	33.9%	4639/12212 (38.0%)	Ongoceng	3.5	20.6%	2271/4205 (54.0%)
Walukuba West	0.7	27.3%	3908/9843 (39.7%)	Aboko	5.5	15.1%	1838/3089 (59.5%)
Walukuba East	0.5	20.9%	2917/7535 (38.7%)	Adyeda	5.7	13.7%	1464/2797 (52.3%)
Bugembe	4.2	4.4%	708/1590 (44.5%)	Apire	6.2	8.4%	933/1712 (54.5%)
Mpumudde	3.6	2.0%	303/727 (41.7%)	Alira	8.6	8.0%	880/1632 (53.9%)
Mafubira	4.7	1.6%	270/586 (46.1%)	Abany	6.6	7.5%	859/1535 (56.0%)
Central Jinja East	1.9	1.1%	206/388 (53.1%)	Atongtidi	12.4	3.6%	361/726 (49.7%)
Others**	N/A	8.9%	1440/3198 (45.0%)	Anwangi	11.0	2.8%	280/578 (48.4%)
				Inomo	10.4	2.7%	269/553 (48.6%)
				Abedmot	13.3	1.7%	150/353 (42.5%)
				Agwiciri	13.0	1.6%	179/316 (56.7%)
				Akali	12.9	1.4%	144/290 (50.0%)
				Aornga	14.0	1.4%	129/289 (44.6%)
				Ajok	16.4	1.4%	116/280 (41.4%)
				Acaba	15.2	1.1%	104/224 (46.4%)
				Abedi	14.3	1.1%	111/222 (50.0%)
				Etekober	15.2	1.1%	100/216 (46.3%)
				Others**	N/A	7.0%	618/1431 (43.2%)
Total	**N/A**	**100%**	**14391/36079 (39.9%)**	**Total**	**N/A**	**100%**	**10806/20448 (52.9%)**

* Distance from center of parish to sentinel site health facility in km.
** Combinations of all parishes with frequencies < 1%.

pLDH-based RDTs. All trends for observed and expected TPR move in the same direction each month over the entire study period. However, it is notable that the expected trends in TPR with RDTs are flatter than trends in observed TPR, most obviously in the case of expected TPR with HRP-2-based RDTs at Aduku. For example, the highest and lowest values, which occurred in September 2009 and December 2010, respectively, were 69% and 34% for the observed TPR and 88% and 75% for the expected TPR with the HRP-2-based RDT.

Discussion

TPR is increasingly used as an indicator of temporal trends in malaria morbidity. Ideally, changes in TPR over time will reflect true changes in malaria incidence for a population of interest. However, several factors including potential confounders such as age and area of residence, proportion of cases subjected to testing, care-seeking and utilization trends, and choice of diagnostic test may cause changes in TPR independent of true changes in the incidence of malaria. In this study, the effects of age, area of residence, and diagnostic test on TPR were investigated at two sites with different transmission intensity in Uganda. Age and area of residence demonstrated the potential to be important confounders at both sites given their independent associations with both time and TPR. However, controlling for each of them had only a small effect on the trends in TPR at the two sites. The directions of trends in the expected TPR using pLDH- and HRP-2-based RDTs were similar to trends in observed TPR, but there were differences between the values of observed and expected TPR at all time points. These differences were more pronounced at Aduku, the higher transmission site, and more pronounced using the HRP-2-based RDT.

Potential confounders are an important consideration in observational studies assessing any association, including temporal trends, which represent associations between time and an indicator of interest, in this case TPR. Any factor associated with both the exposure of interest (calendar time) and the outcome of interest (TPR) has the potential to confound temporal trends in TPR. Numerous factors may be associated with both time and malaria incidence such as weather, precipitation patterns, proportion of patients tested, care-seeking and utilization and home construction. Age and area of residence were chosen for analysis because they are well known to be associated with malaria incidence, and they can both be easily measured.

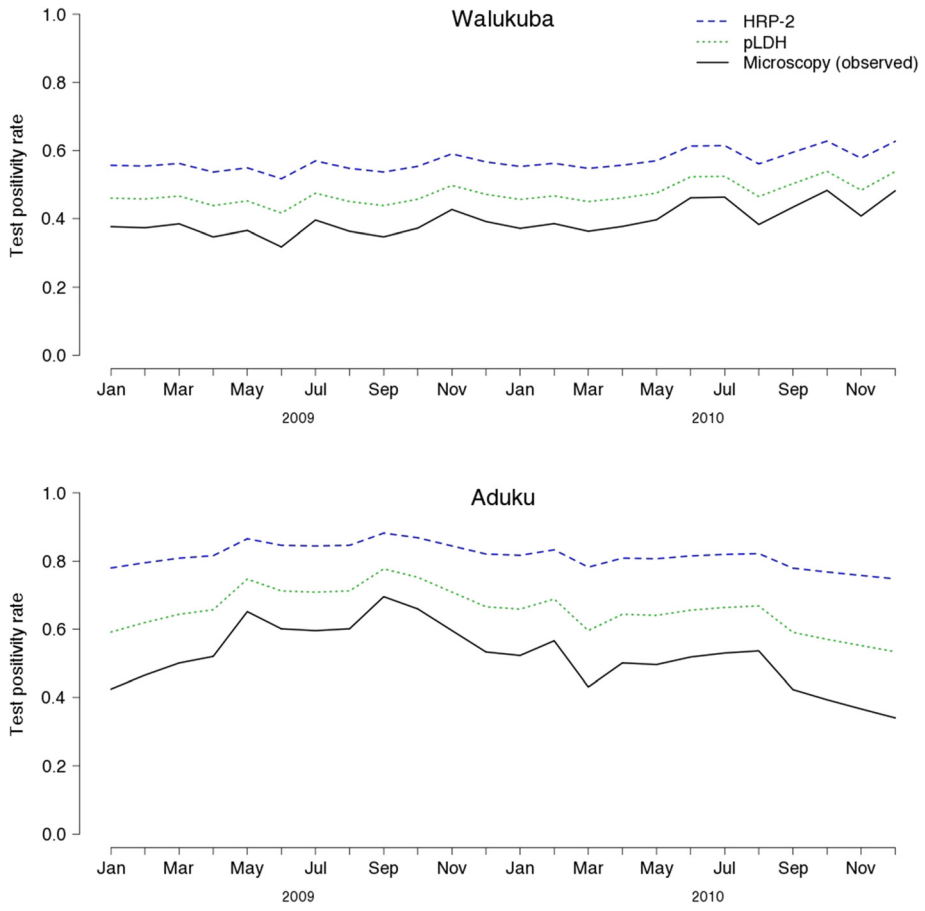

Figure 2 TPR with microscopy (observed) and expected TPR with pLDH- and HRP-2-based RDTs (TPR = test positivity rate).

In the case of age, the expected association between younger age and higher TPR was observed. The age distribution of patients also differed significantly by calendar time, particularly at Aduku where the population receiving a malaria blood smear became gradually older over time. The comparison of temporal trends in observed TPR and age-adjusted TPR in Aduku provides a subtle demonstration of confounding in which age-adjusted TPR increased over time relative to the observed TPR due to the gradual increase in age over time and the lower TPR in older patients. Although confounding by age only had a modest effect on temporal trends in TPR in this study, the effect could be larger in other circumstances, for example a large increase in paediatric capacity at the clinic where surveillance is being conducted.

TPR was also associated with area of residence, though there were no clear patterns relative to distance from the clinic. In the case of Aduku, the TPR was lower outside the catchment area compared to near the clinic, whereas the opposite was true in Walukuba. As with the age distribution, the distribution of area of residence for patients receiving a blood smear also varied over time at both sites. However, there was no noticeable confounding of temporal trends in TPR by area of residence at either site. Nonetheless, it is easy to imagine circumstances in which confounding of temporal trends in TPR by area of residence may be important such as changes in the availability of transportation to the clinic from one area relative to another with a substantially different malaria burden. Controlling for factors such as age and area of residence with methods such as direct standardization or stratification can assure that changes in TPR over time that are due to confounding by these factors are not mistakenly ascribed to changes in malaria morbidity.

The choice of diagnostic test can also affect the interpretation of trends in TPR in two important ways. First, even when the proportion of patients with true infections stays the same, a change from one diagnostic test to another could cause a change in TPR that is exclusively due to a change in the proportion of true positive and false positive tests. Separately reporting the TPR for microscopy and RDTs, as is done in the World Malaria Report [2], partially addresses this problem. However, it still would not account for a substantial change in the quality of microscopy, which can be widely variable

[15,16], or a change from one RDT to another with different sensitivity and specificity [14]. Second, the choice of diagnostic test affects the slope of the trends in TPR, and therefore the ability to distinguish a real difference in malaria morbidity. A low specificity test, which generates more false positives, will tend to obscure trends within the upper range of TPR values (closer to 1), whereas a low sensitivity test, which generates more false negatives, will obscure trends within the lower range of TPR values (closer to 0). High transmission sites such as Aduku are more likely to have a higher TPR, and are more likely to suffer from decreased specificity of RDTs, presumably due to frequent infections and persistence of parasite antigens after resolution of infection [14]. This effect is demonstrated in the comparison between a relatively large decrease in observed TPR at Aduku between September 2009 and December 2010 and a much smaller decrease in the expected TPR with an HRP-2-based RDT (Figure 2). These two effects of diagnostic test on trends in TPR can be accounted for by calculating the sensitivity and specificity of diagnostic tests periodically *via* comparison with a gold standard at the health facilities of interest. Using those results, the TPR can be adjusted accordingly based on the equation shown earlier.

This study has several important limitations. First, the sensitivity and specificity of RDTs at these sites using microscopy as a gold standard may have changed between the time of the study referenced for those values [14] and this study. Sensitivity and specificity of RDTs have been reported to vary based upon clinical and epidemiologic setting, most often related to differences in the distribution of parasite densities among infected patients, and based upon changes in storage and usage of the tests [17]. Such a change may have affected the magnitude of differences between the values and slopes of observed and expected TPR, but the general direction of those differences likely would have been the same. Second, given the large samples sizes in this study, it is not surprising that statistically significant differences were found between potential confounders and the exposure (calendar time) and outcome (TPR) of interest. Indeed, the magnitude of these differences were of questionable relevance in terms of potential confounding and temporal trends in TPR based on adjusted analyses did not reveal differences compared to the unadjusted temporal trends that would likely be of public health importance. Third, this study was limited to two sentinel surveillance sites in Uganda, and may not be representative of many areas of the world with lower transmission intensity. TPR is not very useful as a surveillance indicator in settings with very low transmission intensity, though a TPR below 5% has been recommended as one of the criteria for readiness to shift to the elimination phase of malaria control [2]. Finally, even in settings with malaria transmission on the order of that in these two study sites, TPR has many important limitations as an indicator of malaria burden which have been discussed elsewhere - the numerical change in TPR does not reflect either linear or proportional changes in malaria incidence in the population sampled, it is useful to estimate relative changes in malaria incidence but it cannot be used to estimate the actual incidence or compare incidence across sites, and changes in its value could be caused by changes in non-malarial fevers, the population of patients accessing the health facility, or changes in testing practices at the health facility [9].

Conclusions

TPR is a key malaria surveillance indicator in resource-limited settings with medium to high transmission. It is easily integrated into HMIS reporting, and its reliability will depend less upon stable clinic attendance as do estimates of malaria incidence based on clinical or laboratory diagnosis, so long as a consistently high and representative proportion of clients access health facilities and are offered a diagnostic test. A thorough understanding of both the limitations of TPR and methods for improving its accuracy is important for monitoring the effectiveness of malaria control interventions. Indeed, improved methods to quantify and compare changes in TPR in different settings could be used to provide more practice-based evidence on the relative effectiveness of malaria control interventions. The urgency of the overall burden of malaria, the increasing availability of new tools to fight malaria, and the tremendous resources required for controlled experiments of public health interventions demand creative techniques such as health facility-based surveillance with indicators like TPR to efficiently test, refine, and deploy the next generation of strategies for malaria control.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions
AG, RK, SPK, SN, MRK and GD contributed to study design and oversight. DF, AG, and GD contributed to methodology, data analysis, interpretation of results, and drafting of the manuscript. All authors read and approved the final manuscript.

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COMPARISON OF HRP2- AND pLDH-BASED RAPID DIAGNOSTIC TESTS FOR MALARIA WITH LONGITUDINAL FOLLOW-UP IN KAMPALA, UGANDA

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Abstract. Presumptive treatment of malaria results in significant overuse of antimalarials. Malaria rapid diagnostic tests (RDTs) may offer a reliable alternative for case management, but the optimal RDT strategy is uncertain. We compared the diagnostic accuracy of histidine-rich protein 2 (HRP2)- and plasmodium lactate dehydrogenase (pLDH)-based RDTs, using expert microscopy as the gold standard, in a longitudinal study of 918 fever episodes over an 8-month period in a cohort of children in Kampala, Uganda. Sensitivity was 92% for HRP2 and 85% for pLDH, with differences primarily due to better detection with HRP2 at low parasite densities. Specificity was 93% for HRP2 and 100% for pLDH, with differences primarily due to rapid clearance of pLDH antigenemia after treatment of a previous malaria episode. RDTs may provide an effective strategy for improving rational delivery of antimalarial therapy; in Kampala, either test could dramatically decrease inappropriate presumptive treatments.

INTRODUCTION

Diagnostic capabilities are limited in Africa, and in most cases fevers are treated presumptively as malaria without laboratory-confirmed diagnosis. In many settings, presumptive treatment of all fevers as malaria results in extensive overuse of antimalarials and delays the diagnosis of other causes of fever.[1–4] With older antimalarial drugs, which were inexpensive, safe, and widely available, the potential benefits of early treatment of all fevers supported presumptive antimalarial therapy. However, in the era of increasing drug resistance, new combination therapies are being deployed that are much more expensive and have less established safety records.[5,6] In this setting, improved ability to diagnose malaria may prevent many unnecessary antimalarial treatments and should also allow prompt attention to other causes of fever when malaria is ruled out. Light microscopy, for decades the gold standard for malaria diagnosis, remains unavailable to most patients in Africa.[7,8] Malaria rapid diagnostic tests (RDTs), newer diagnostic modalities that identify circulating antigens of malaria parasites, may offer a reliable alternative for case management.

The most studied malaria RDTs offer simple identification of two parasite antigens: histidine-rich protein 2 (HRP2) and plasmodium lactate dehydrogenase (pLDH). HRP2 was the first antigen targeted by an RDT,[9] has been available in various commercial formats for several years, has shown good sensitivity in a variety of field settings, and is increasingly advocated as a diagnostic test where reliable microscopy is not available. A potential problem for HRP2-based assays is persistence of detectable circulating antigen for up to several weeks after parasites have been eradicated.[10–12] Persistent HRP2 antigenemia has not correlated with treatment failure, suggesting that this finding is not representative of persistent infection.[10,12] Persistent antigenemia thus may limit the usefulness of HRP2-based assays in areas of intense malaria transmission, where positive tests may commonly be due to prior infections that are no longer clinically relevant. pLDH-based RDTs appear to be slightly less sensitive than those detecting HRP2, but the antigen is rapidly cleared from the bloodstream, becoming undetectable at about the same time blood smears become negative after antimalarial therapy.[13–15] Thus, if sensitivity is adequate, the increased specificity of pLDH-based assays for acute malaria suggests that they may be better-suited for high-transmission areas, such as much of sub-Saharan Africa. With increasing advocacy for the implementation of RDTs, it is critical that optimal diagnostic strategies are identified. The true impact of the varied sensitivity and specificity of different tests is best compared with long-term follow-up to consider the impacts of prior infections and persistent antigenemia on test results. For this reason, we compared the diagnostic accuracy of HRP2- and pLDH-based RDTs, using expert microscopy as the gold standard, in a longitudinal cohort of children in Kampala, Uganda.

METHODS

Study population and longitudinal drug-efficacy trial. We evaluated two RDTs in a cohort of 601 children enrolled in an on-going longitudinal antimalarial treatment efficacy trial in Kampala. The trial began in November 2004, and is based at Mulago Hospital, Uganda's main public hospital. Participating children are residents of Mulago III parish, located within 2 km of Mulago Hospital. Households were randomly selected for enrollment into the trial after a census of the parish.[16] Children in the study cohort receive all their medical care free of charge at our study clinic. Participants are encouraged to come to the clinic promptly for any illness and to avoid any medications not administered by study clinic staff. Participants are seen at least monthly, either at the study clinic for evaluation of illness or for routine follow-up visits, or during home visits. Each time a participant presents to the study clinic with fever (documented tympanic temperature $\geq 38°C$ or history of fever within the previous 24 hours), a fingerprick blood sample is obtained for thick and thin smears and storage on filter paper. If the blood smear is positive, the child is treated with antimalarials and followed for 28 days; if the smear is negative, the child does not receive antimalarials and is treated according to standard clinical algorithms and

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the study physician's judgment. Parents/guardians gave informed consent for all study procedures, and the study was approved by the Uganda National Council of Science & Technology and by the institutional review boards of Makerere University and the University of California, San Francisco.

RDT study methods. At the time of the RDT evaluation, children in the cohort ranged in age from 1.5 to 11.5 years. From October 2005 to May 2006, each time a blood smear was done to evaluate fever in a study participant, except when the fever occurred within 3 days of a confirmed episode of malaria, a fingerprick blood sample was obtained for the two RDTs in addition to thick and thin smears and storage on filter paper (Figure 1). If a participant presented with repeated episodes of fever after diagnosis of a non-malarial illness, the RDT was repeated at the study physician's discretion. Clinical management was guided by microscopy results; RDT results did not influence patient care.

Thick and thin smears were stained with 2% Giemsa for 30 minutes and read by experienced laboratory technologists. Parasite densities were calculated from thick smears by counting the number of asexual parasites per 200 leukocytes (or per 500 leukocytes, if the count was < 10 asexual parasites/200 leukocytes), assuming a leukocyte count of 8,000/μL. Smears were considered negative if the examination of 100 high-power fields did not reveal asexual parasites. Gametocytemia was determined from thick smears and parasite species from thin smears. All smears were read a second time by study laboratory staff to confirm results, and discrepant readings were resolved by a third reader. If the first and second readers both reported a positive smear, but the second density report differed from the first by ≥ 2000/μL, the final density recorded was that of the third reader.

RDTs were selected for evaluation on the basis of ease of use (relatively few preparation steps and clear distinction between positive and negative results), safety (minimal exposure to blood during test preparation), completeness of packaging and labeling, appropriate packaging for transport and storage in tropical environments (each test individually wrapped in foil with plastic liner and desiccant), low market price, and reliability of supply. The RDTs studied were Paracheck (detection of HRP2, Orchid Biomedical Systems, Goa, India) and Parabank (detection of pLDH, Zephyr Biomedicals, Goa, India). RDTs were obtained directly from the manufacturers and stored in their original packaging at room temperature in the study clinic. Temperature and humidity of the storage area were monitored, but not controlled. Over the course of the study period, the temperature in the storage area ranged from 19 to 29°C, with a mean low of 24°C and a mean high of 27°C. The relative humidity ranged from 31% to 82%, with a mean low of 53% and a mean high of 69%. Prior to the beginning of the study, positive and negative control blood samples were obtained, and stored at −80°C for quality-control testing of RDTs throughout the study. Each batch of RDTs underwent quality-control testing when opened and at 8- to 12-week intervals thereafter. The two positive control samples had densities 84/μL and 5000/μL, respectively. All

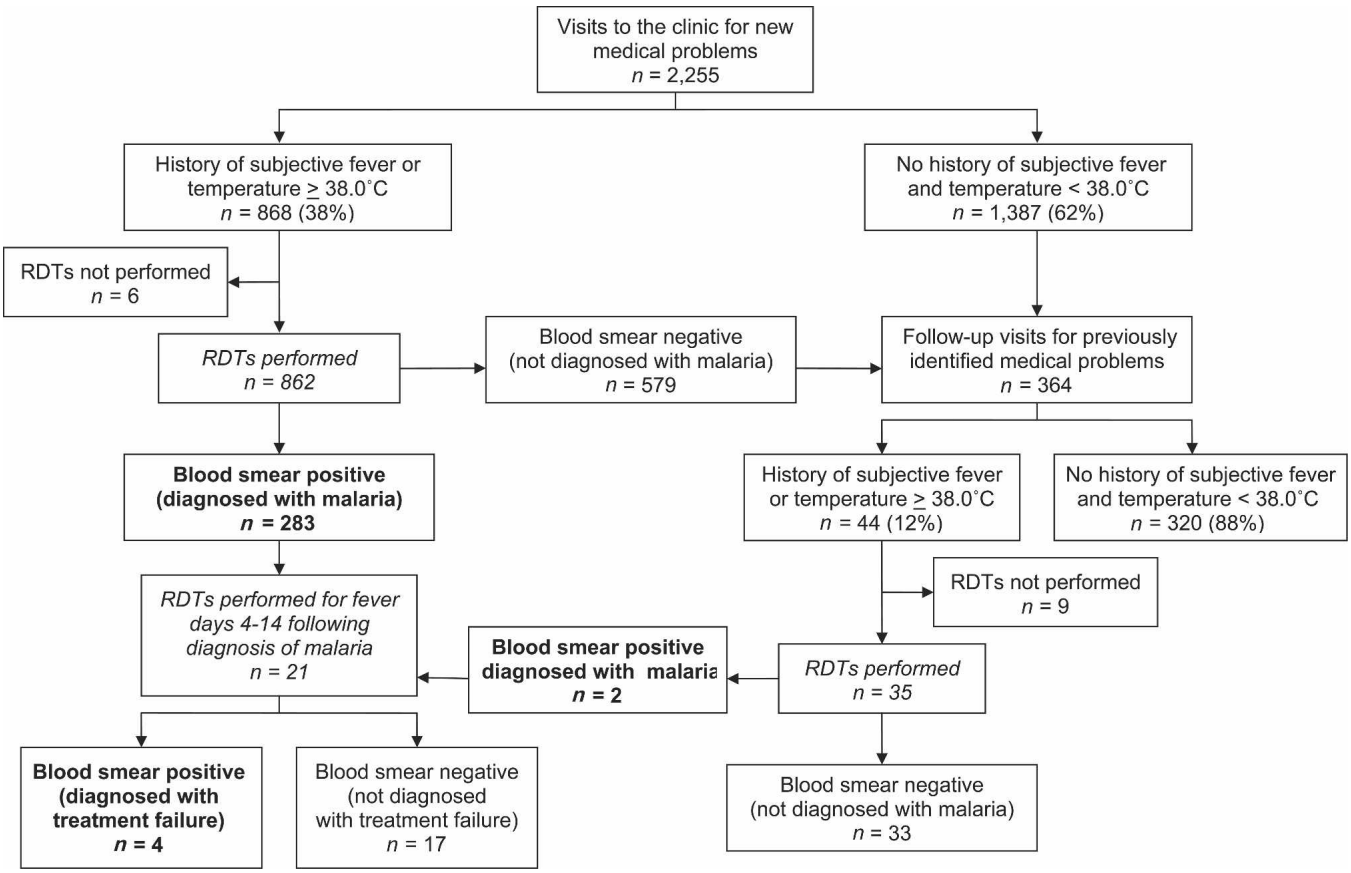

FIGURE 1. Trial profile showing clinic visits, blood smear results, rapid diagnostic tests (RDTs) performed (italics), and malaria episodes (bold). At the beginning of the RDT evaluation, 565 children were enrolled in the study cohort; 524 remained enrolled at the end of the evaluation.

HRP2 RDTs tested with quality-control samples were accurate; all pLDH RDTs tested were accurate for the negative and 5000/μL samples, but only 2 of 8 were accurate for the 84/μL sample.

RDTs were prepared and read by study physicians and then read by laboratory technicians. All readers were trained to perform the tests according to manufacturers' instructions. Study physicians interpreted and recorded RDT results after 15 minutes, at which time they were unaware of blood smear results. They were advised that if the background of the RDT test window remained pink (bloody) at the end of 15 minutes, they should allow the background to clear before reading the RDT. RDTs were then carried to the adjacent study laboratory, where they were re-read by laboratory technicians who were unaware of both the physician's interpretation and the patient's microscopy result. Readers recorded RDT results as either positive or negative; they were trained to consider faint test lines as positive.

Molecular methods. PCR was performed to identify parasite species in samples positive by microscopy but negative by RDT, as well as to detect subpatent infections in samples negative by microscopy but positive by RDT, and in a random sample of microscopy-negative and RDT-negative samples. DNA was extracted from filter paper samples using Chelex resin[17] and stored at −20°C until use. To detect *Plasmodium falciparum*, the block-3 region of merozoite surface protein-2 (msp-2) was amplified by nested PCR with primers corresponding to conserved sequences flanking this region[18] followed by primers to amplify the *IC3D7* and *FC27* allelic families, using conditions described previously.[19] In addition, to detect *P. falciparum, P. vivax, P. malariae*, and *P. ovale*, genus-specific followed by nested species-specific PCR of 18S small subunit ribosomal RNA[20] (ssu rRNA) for the four species (Malaria Research and Reference Reagent Resource Center, Manassas, VA) was performed, using oligonucleotide primers and conditions as described previously.[21] PCR products were analyzed by electrophoresis using 2% agarose gels.

Statistical methods. Data were entered using Epi-Info version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA) and analyzed with Stata version 8.0 (Stata Corporation, College Station, TX). Sensitivity, specificity, and positive and negative predictive values were calculated by comparing the proportion of positive and negative results for each RDT with expert microscopy. Categorical variables were compared using χ^2 or Fisher's exact test. A P value of < 0.05 was considered statistically significant.

RESULTS

Overall RDT accuracy. We evaluated 918 episodes of fever over an 8-month interval in children from our cohort in Kampala (Figure 1). Over the 8-month period, 868 fevers were new fevers in a previously well child, 21 occurred 4–14 days after a diagnosis of malaria, and 44 occurred during follow-up of a non-malarial illness. RDTs were not performed in 15 episodes, in 9 at the discretion of the physician during follow-up of a non-malarial febrile illness, and in 6 because of protocol errors. Light microscopy identified positive malaria smears in 289 episodes (31%). Blood smear results served as the gold standard for comparison with RDT results. As RDT results are dependent on reader accuracy, we compared readings by two groups of clinic personnel: study physicians and laboratory technicians. In both cases, the sensitivity (> 92%) and negative predictive value (> 96%) were higher for the HRP2 assay, and specificity (> 99%) and positive predictive value (> 99%) were higher for the pLDH assay (Figure 2). First readers interpreted RDT results an average of 15 minutes after preparation, and second readers interpreted results an average of 7 minutes later. First and second test readings agreed in 98% of readings; they disagreed for 16 HRP2 tests and 13 pLDH tests. For 14/16 (88%) discordant HRP2 readings and 10/13 (77%) discordant pLDH readings, only second readings were positive.

Evaluation of false-negative results. Possible reasons for false-negative RDT results include low parasite density, non-falciparum parasite species, and interpreting the RDT before the test line has fully developed. HRP2 is produced only by *P. falciparum* parasites, while the pLDH assay evaluated here detects antigen from all human malaria parasites, although some reports suggest pLDH may be less sensitive for non-falciparum species.[22,23]

Of the 22 false-negative HRP2 results (based on first reading), 15 (68%) occurred in non-falciparum infections (Figure 3). Of the remaining 7 false negatives, 5 were interpreted as positive by the second reader. The 2 remaining false negatives occurred in a *P. falciparum* mono-infection with parasite density 48/μL, and a *P. falciparum* and *P. vivax* mixed infection with density 680/μL. Considering only *P. falciparum* infections, the sensitivity of the HRP2 assay at the second reading was 99% (272/274).

Of the 43 false-negative pLDH results, 12 (28%) occurred in non-falciparum infections; the remaining 31 were all *P. falciparum* mono-infections. Of these 31 false negatives, 9 were interpreted as positive by the second reader. For the remaining 22 false negatives, the geometric mean parasite density was 352/μL (range 16 to 26,080/μL). Considering only *P. falciparum* infections, the overall sensitivity of the pLDH assay at the second reading was 91% (250/274). The sensitivity for *P. falciparum* infections decreased from 98% (217/222) to 88% (28/32) to 25% (5/20) for parasite densities > 5000/μL, between 1000 and 5000/μL, and ≤ 1000/μL, respectively ($P < 0.0001$).

Evaluation of false-positive results. Possible reasons for false-positive RDT results include persistent antigenemia after antimalarial treatment, detection of gametocytes when asexual forms are not present, RDT detection of low-density microscopy-negative infections, or presence of antigenemia early in infection before parasites are detected by microscopy.

Of the 42 false-positive HRP2 results, 12 (29%) occurred within 14 days of a prior diagnosis of malaria, 26 (62%) within 28 days, and 32 (76%) within 42 days. In contrast, negative HRP2 results occurred as early as 7 days after initial diagnosis of a previous episode of malaria.

Gametocytes were detected by microscopy in only 12 of the 918 cases (1.3%). No HRP2 result was positive in a case where the smear showed gametocytes but not asexual parasites.

PCR was conducted to assess whether false-positive RDT results may have been associated with subpatent parasitemia. Of 40 evaluable false-positive HRP2 results, PCR was positive for *P. falciparum* in 8 (20%), compared with PCR positivity in 5/66 (8%) of control HRP2- and microscopy-negative samples ($P = 0.07$). Four of the 8 smear-negative, RDT- and

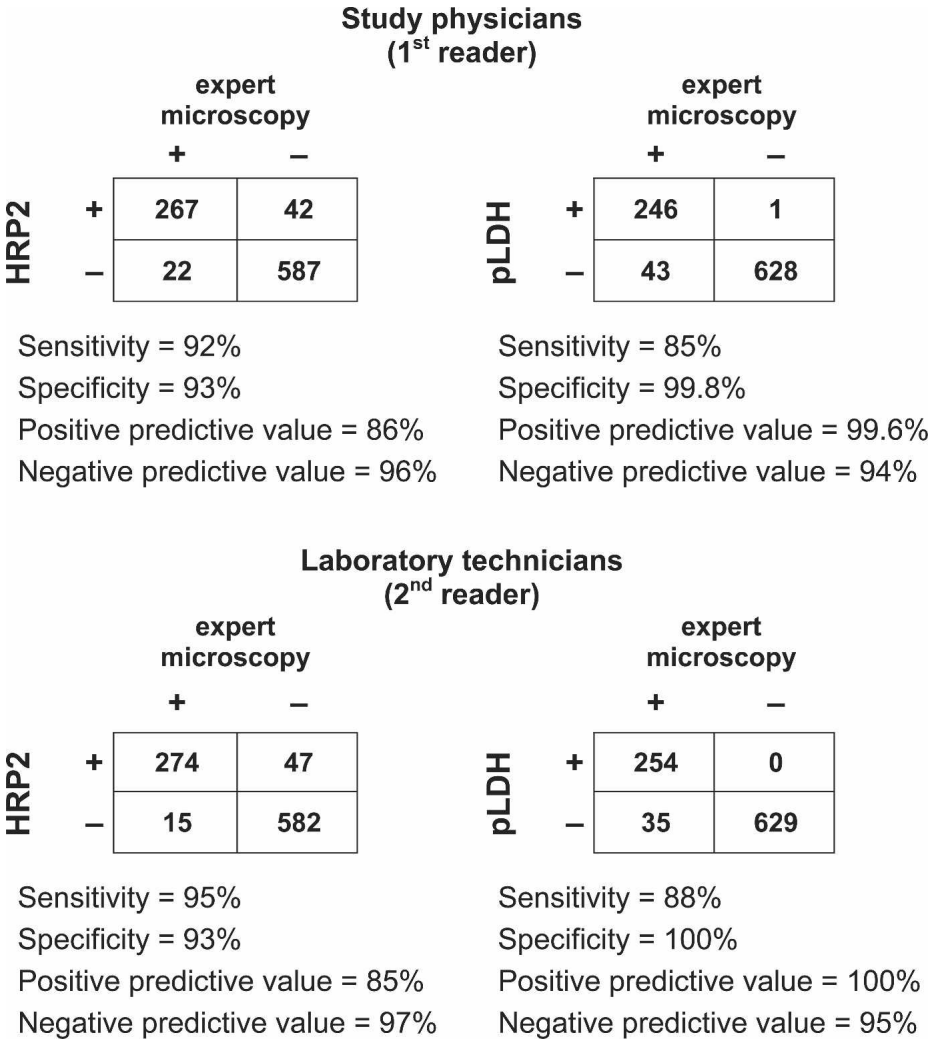

FIGURE 2. Point estimates of RDT accuracy. Blood smears were read by experienced microscopists in the study laboratory. All smears were read a second time by study laboratory staff to confirm results, and discrepant readings were resolved by a third reader. RDTs were read sequentially by study physicians and laboratory technicians, as described in Methods.

PCR-positive samples were obtained within 28 days of a prior episode of malaria.

Negative HRP2 results were recorded up to 3 days prior to an episode of malaria. Only one patient developed malaria within a week after a false-positive HRP2 result. The sample from the initial evaluation showed no asexual parasites or gametocytes but was positive for *P. falciparum* by PCR. The patient returned with persistent fever 5 days after initial evaluation, at which time the blood smear was positive, with parasite density 52,840/μL.

Only one pLDH test result was false-positive, in a patient who had no documented previous episode of malaria over 469 days of follow-up, and no malaria during the subsequent 2 months of study follow-up. No gametocytes were seen in the smear, the sample was negative by PCR for all four malaria species, and the second reading of the RDT was negative, strongly suggesting that this false positive was due to an error during the first test reading.

DISCUSSION

As compared with microscopy, both HRP2- and pLDH-based RDTs demonstrated acceptable sensitivity and specificity for the diagnosis of malaria in Kampala. The HRP2 assay showed superior sensitivity but inferior specificity compared with the pLDH assay. The longitudinal design of our study allowed us to clarify the relative importance of contributors to RDT false-negative and false-positive results. The difference in sensitivity between the tests was due mostly to better detection with HRP2 at low parasite densities. Non-falciparum infections contributed to false-negative results for both RDTs. In particular, in two-thirds of cases in which the HRP2 test was negative although microscopy detected parasites, the infection was caused by non-falciparum species. The higher specificity and positive predictive value of the pLDH assay was due to the fact that pLDH antigenemia closely mirrors parasitemia, while HRP2 commonly persists in the bloodstream weeks after successful treatment of malaria.[10,12] Subpatent parasitemia, as detected by PCR, pre-patent infections, and gametocytemia, did not appear to contribute importantly to false-positive results for either RDT. In summary, both studied RDTs accurately identified clinically relevant malaria infections but they differed importantly in sensitivity and specificity.

In Uganda, RDTs are increasingly available in the private

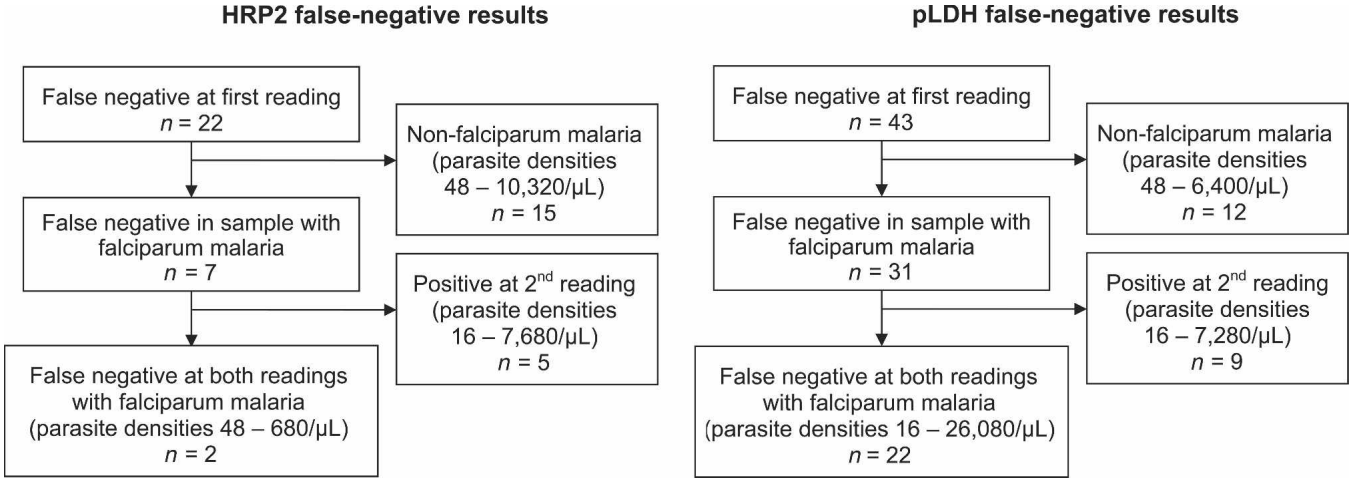

FIGURE 3. Factors associated with false-negative HRP2 and pLDH RDT results.

health care sector and are widely advocated for use in the public sector, though clear guidelines or algorithms for their use are lacking. In Kampala, both the HRP2 and pLDH tests showed a high negative predictive value and appeared to offer good reliability in ruling out malaria as the cause of a fever. Considering the potential values of RDTs, some limitations in both sensitivity and specificity may be acceptable. The lower specificity of the HRP2-based test, due to persistent antigenemia after recent infections, may lead to some inappropriate treatments, but many fewer than if all episodes of fever were treated as malaria. However, the lower specificity of HRP2 assays may be more problematic, with many more unnecessary malaria treatments, in regions with higher transmission intensity than Kampala. The lower sensitivity of the pLDH-based assay might also be a concern, but in Kampala, missed episodes were primarily of relatively low parasitemia, suggesting that, in immune populations, mostly mild or asymptomatic infections will be missed. Indeed, especially if technological innovations can improve the sensitivity of pLDH-based tests, they may well offer the optimal balance of sensitivity and specificity for the diagnosis of malaria in Africa.

To our knowledge, this study offers the first comparison of RDTs in a longitudinal format, allowing assessment of the importance of previous and future malaria infections in RDT accuracy. A number of other RDT evaluations have been conducted, though results have varied widely, likely due at least in part to different methodologies and locations. Two previous RDT studies in western Uganda compared HRP2-based tests with expert microscopy. One evaluation, using an older HRP2 assay, found a sensitivity of 99.6% for parasitemia > 500/μL and specificity of 92.7% in patients with fever.[24] The other study, using the same HRP2 test as in our evaluation, found a sensitivity of 97% and specificity of 88% for *P. falciparum* infections.[25] These estimates are similar to those for the HRP2-based test in our current evaluation. Our results also confirm the superior specificity of pLDH seen in a study in Tanzania,[26] although sensitivity of both tests was somewhat lower in our study.

Our results are not immediately applicable to fever case management across Africa. We obtained RDTs directly from manufacturers, and we used and stored kits as recommended by manufacturers; adherence to these guidelines may be challenging in rural settings, and test quality is likely to deteriorate if kits are less well maintained.[27] Our evaluation was performed in an area with relatively low malaria transmission. Because of the location and design of our study, our patients likely presented to the clinic earlier in the course of malaria than in non-research settings. Our staff was carefully trained in use of the two RDTs before initiation of our study; test accuracy may be lower in field settings, although a number of reports indicate that village health workers with minimal training are able to satisfactorily prepare and interpret RDTs.[28,29] Considering these limitations, how should the results of this evaluation influence malaria treatment policy? For Kampala, our results suggest that, in settings without access to microscopy, use of either HRP2- or pLDH-based RDTs could dramatically lower the use of inappropriate antimalarial therapy without missing many episodes of clinical malaria. However, it will be necessary to perform similar analyses in areas with different epidemiology to determine the predictive values of different RDTs in various settings. In addition, the issue of cost and cost-effectiveness of RDTs, compared with presumptive treatment and with diagnosis with microscopy, must be considered. In the era of artemisinin combination therapies, using RDTs to target treatment to confirmed cases of malaria may help to maximize the impact of these valuable resources.

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Point-of-care tests for malaria

Horizon Scan Report 0040
January 2015

Clinical Question:

In the primary care setting, what is the accuracy and utility of malaria point-of-care (POC) tests in the detection of parasitaemia caused by *Plasmodium* species, compared to standard laboratory practice using Microscopy and/or Polymerase Chain Reaction (PCR)?

Background, Current Practice and Advantages over Existing Technology:

Background:

Malaria is an important infectious disease, caused by the protozoan *Plasmodium* and transmitted by inoculation with an infected Anopheles mosquito. A variety of *Plasmodium* species cause malaria, typically producing cyclical systemic symptoms including fever, headache, vomiting and lethargy. Infection with *Plasmodium falciparum* can result in severe disease, and can lead to neurological sequelae including cerebral malaria and at worst death.

The World Health Organisation (WHO) World Malaria Report of 2009 estimates 243 million cases of malaria worldwide in 2008, the majority of which (85%) occurred in Africa, followed by South-East Asia (10%) and then the Eastern Mediterranean (4%).(**1**)

Whilst the largest burden of disease rests in Africa, the burden of malaria is increasing in non-endemic, industrialized areas due to imported disease in returning travellers who have no immunity (**2**). Many travellers do not comply with use of appropriate chemoprophylaxis and insect protection measures (**3**). For the reasons outlined above, malaria is an important differential diagnosis in febrile patients who have travelled to malaria endemic regions.

Current Practice and Advantages over Existing Technology:

a) **Primary care assessment of patients with suspected malaria**
   Existing Technology: Patient is clinically reviewed by General Practitioner (GP) and if malaria is suspected, liaison takes place with Infectious Diseases Registrar/medical registrar, with subsequent assessment of the patient in an Infectious Diseases Unit or appropriate Medical Assessment Unit. It is unlikely that blood samples would be sent from General Practice, due to the time delay that this would incur. However, were this to take place, blood samples would be sent from General Practice to the local hospital laboratory for analysis of thick and thin blood films for *Plasmodium* forms. Results would typically be sent back to the GP within

24 hours. Depending on the significance of the result, this may or may not need to be relayed to the Infectious Diseases Registrar and hospital admission planned.

Benefits of malaria POC testing: Rapid (within minutes) positive or negative malaria result, expediting referral to the Infectious Diseases team if positive, and investigation of other causes of febrile illness if negative without referral to the Infectious Diseases team. This technology could therefore allow assessment to move from a secondary care setting to primary care. This may lower testing thresholds.

b) Secondary care assessment of patients with suspected malaria

Existing Technology: Patients with suspected malaria in secondary care are frequently managed on Infectious Diseases wards and have an EDTA blood sample taken and analysed in the hospital laboratory. Here, the specimen is analysed under a microscope for *Plasmodium* forms. A diagnosis and/or level of parasitaemia is then estimated and appropriate treatment commenced if necessary. Other tests, such as PCR, may also be employed as a reference test.

Benefits of malaria POC testing: Rapid (within minutes) result of malaria infection, allowing prompt initiation of appropriate treatment. POC tests can be used in conjunction with microscopy, the latter helping to identify the specific *Plasmodium* species so as to direct treatment.

Details of Technology:

Malaria POC tests are generally portable, hand-held devices, the majority of which employ lateral-flow immunochromatography to detect *Plasmodium* antigens in a finger-prick sample of blood. A positive or negative result can be generated in as little as 10 minutes, allowing rapid diagnosis or exclusion of malaria. Their rapidity and also simplicity of use, not requiring specialist knowledge or equipment, are seen as their principle advantages over the current gold standard of laboratory based microscopy of thick and thin blood films.

Malaria POC tests can be grouped largely on the basis of the *Plasmodium* antigen detected. Some tests detect histidine-rich protein (HRP-2), which is solely produced by *Plasmodium falciparum*. Other tests detect aldolase, which is common to all *Plasmodium* species and therefore pan-specific. Yet other tests detect parasite lactate dehydrogenase enzymes (pLDH), which can be pan-specific, targeting a conserved pLDH element found in all *Plasmodium* species, or specific to particular *Plasmodium* species, targeting species unique regions of pLDH. A summary of available point-of-care malaria tests we identified can be found in the table in Appendix 1.

Patient Group and Use:

1) Ruling out malaria in travellers returning from malaria endemic regions with febrile illness.

2) Ruling out malaria in patients visiting the UK from malaria endemic regions presenting unwell to primary and/or secondary care.

Importance:

Light microscopy is considered the gold standard for malaria diagnosis (**4**). However, microscopic diagnosis of malaria requires time, trained personnel, and adequate laboratory facilities. In many parts of rural Africa in which malaria is most prevalent, access to such services is difficult or simply not possible. As such, there has been considerable interest in developing a new technology that could be used to rapidly diagnose malaria by non-skilled personnel (**5**).

Despite the burden of malaria being considerably less in the United Kingdom, there were 1501 cases of malaria in the UK in 2013 and 7 deaths (**6).** Prompt diagnosis and treatment of malaria could reduce morbidity and mortality. In the primary care setting, laboratory microscopic analysis of blood films is not possible. Implementation of a reliable malaria POC device could facilitate primary care diagnosis of malaria, allowing faster referral to secondary care, and more rapid administration of potentially life-saving treatment where appropriate.

Previous Research:

Accuracy compared to existing technology

Given the topical nature of malaria POC tests, a vast number of studies have examined their accuracy and potential utility. Below, we have focussed on the data from pertinent meta-analyses and other relevant studies.

<u>POC tests in malaria endemic regions</u>

A 2011 Cochrane review (**7**) analysed the use of POC tests in detecting clinical *Plasmodium falciparum* malaria in patients presenting to ambulatory healthcare centres in malaria endemic regions. The reference standard was defined as falciparum parasitaemia detected on microscopy, in conjunction with symptoms suggestive of malaria. Data from 74 studies described in 79 study reports were analysed. The POC tests were divided into seven different categories ('Type 1 tests' through to 'Type 7 tests') dependent on the test target antigen.

The vast majority of tests evaluated were 'Type 1 tests' evaluating HRP-2 specific POC tests. The authors identified 71 evaluations, in which 10 different brands of Type 1 POC tests had been verified with microscopy, encompassing 40,062 individuals. The sensitivities of the tests ranged from 42% to 100%, with specificities between 65% and 100%. The meta-analytical average sensitivity and specificity (95% confidence interval (CI)) were 94.8% (93.1% to 96.1%) and 95.2% (93.2% to 96.7%) respectively. Comparison of the 10 POC test brands analysed did not reveal statistically significant differences (p=0.18), however, substantial heterogeneity between studies was apparent.

There were 17 evaluations of 'Type 4' POC tests (identifying both *Plasmodium falciparum* specific and pan-specific pLDH antigens) verified with microscopy. The meta-analytical average sensitivity and specificity (95% CI) were 91.5% (84.7% to 95.3%) and 98.7% (96.9% to 99.5%), respectively. Upon comparison of the four brands of POC tests used in the type 4 tests evaluations, statistically significant (P=0.009) differences were noted. More precisely, Carestart Malaria Pf/Pan was found be more sensitive but less specific than OptiMAL, OptiMAL-IT and Parabank (sensitivity of 97.8% compared with 90.1%, 87.4% and 87.9%, respectively; specificity of 92.2% compared with 99.3%, 97.0% and 98.8%, respectively).

Statistical comparison was made between 'Type 1' and 'Type 4' tests with significant differences in test accuracy noted (p = 0.009). 'Type 4' tests were found to have a significantly higher specificity (p<0.001) than 'Type 1' tests in the comparisons based on all data, however, no significant difference was found between the sensitivity of these tests (p=0.34). The lower specificity of Type 1 tests may be due to the use of HRP-2 antibodies, which can give a false positive result in successfully treated cases of *Plasmodium falciparum* malaria, due to persistent antigenaemia. Thus, the choice of which test to employ in clinical practice would depend upon the prevalence of malaria in the affected region and additionally the goal of the test. In primary care, the intention would be to exclude malaria, and as such a test with high sensitivity would be desirable. Conversely, a highly specific test might be required in a secondary care setting to aid decisions regarding initiation of treatment.

A meta-analysis (**4**) examined the role of only the Parasight-F POC test (which had also been included in the Cochrane review) in the detection of *falciparum* malaria. 32 studies from 29 publications were evaluated, comprising 15,359 comprising 15,359 resident and non-resident subjects in a variety of malaria endemic and non-endemic countries. The included studies compared Parasight F against microscopy as a reference standard. Parasight-F demonstrated an overall meta-analytical sensitivity of 90.9% and specificity of 94.3%. The authors conclude that Parasight-F is a valid diagnostic tool that could be used stand-alone or in conjunction with microscopy. However, for any test it is important to recognise that the utility of the test is highly dependent upon the prevalence of malaria in a geographical region. Based on the pooled sensitivity and specificity data, in a region of 60% P. *falciparum* prevalence, the positive predictive value (PPV) would be 96%, with a negative predictive value (NPV) of 87%. However, in a region of 10% P. *falciparum* prevalence, the PPV would be much lower at 64%, conversely, the NPV would be 98%.

<u>POC tests in Pregnancy</u>

Plasmodium falciparum infection during pregnancy can result in severe illness and at worst death of mother and foetus (**8**). In pregnant women malarial parasites express an antigenic variant allowing them to sequester in the placenta, known as placental malaria, rendering microscopic diagnosis of peripheral blood inadequate (**9**). Placental histology is therefore the gold standard for diagnosis of placental malaria. However, placental analysis is only possible after delivery, and as such examination of peripheral blood during pregnancy is current standard practice.

A meta-analysis of 49 studies was performed to assess the accuracy of POC tests and PCR in diagnosis of malaria in pregnancy (**10**). Microscopic analysis of peripheral and placental blood was used as a reference standard, with the latter deemed the more accurate reference standard. The

sensitivity (proportion of microscopy positives in placental blood) detected by POC tests was 81%, versus 72% for peripheral blood microscopy and 94% for PCR analysis. The specificity (proportion of placental blood microscopy negative women) detected by POC tests was 94%, against 98% for peripheral blood microscopy and 77% for PCR.

POC tests in Non-immune travellers to malaria endemic regions

A meta-analysis (**2**) analysed the accuracy of POC tests in diagnosing malaria in non-immune travellers returning from malaria endemic countries, predominantly in Africa, Asia and South/Central America. Twenty-one studies were included, encompassing 5747 patients; eighteen of these studies were performed at regional or national tropical disease centres. The use of HRP-2 based tests and pLDH based tests was compared against microscopy and/or PCR as gold standards. Both two-band (detecting *Plasmodium falciparum* only) and three-band (detecting *Plasmodium falciparum* as well as *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium vivax*) HRP-2 tests were included in the analysis. Studies in which more than 10% of individuals were immune were excluded.

The negative likelihood ratio (LR-) was predefined as the primary measure of accuracy. This meta-analysis found that HRP-2 tests were statistically significantly more accurate than p-LDH based tests at ruling out *Plasmodium falciparum*, with LR-s of 0.08 and 0.13 respectively (p=0.019 for difference). For *Plasmodium vivax*, there was no statistically significant difference between the LR-for three band HRP-2 tests compared to parasite LDH tests (LR-s of 0.24 and 0.13 respectively; p=0.22), however, the available studies upon which these figures were based were few and heterogeneous in nature. The authors conclude that POC tests are a useful to rule out malaria when negative, but they should be used in conjunction with microscopy for species identification and confirmation when positive.

Summary

POC tests appear to be an accurate alternative compared to traditional microscopic analysis of blood films for malarial parasites. POC tests detecting HRP-2 antigens appear to have a higher sensitivity but lower specificity than POC tests detecting p-LDH. As such, the choice of which POC test to employ would largely depend upon the prevalence of malaria in the region of interest and the intended goal of the test. Given that the UK is a non-endemic region largely dealing with malaria in travellers and immigrants from endemic regions, and the aim of any rapid test would be to rule out. It is difficult to specify an optimal time-frame within which POC tests should be used given the varying incubation periods of *Plasmodium* species; in addition, latent blood infection with *Plasmodium* parasites can persist for years.

Impact compared to existing technology

A Cochrane meta-analysis (**11**) reviewed the utility of POC tests versus clinical diagnosis (relying on symptomatology and clinical signs alone) of malaria in febrile patients in rural African endemic settings, with a view to assessing whether this would reduce inappropriate use of anti-malarial drugs in patients with febrile illness not caused by malaria. Seven trials were reviewed, consisting of 17,505 febrile patients. Overall, POC tests did not reduce the number of unwell patients at day 4-7

post treatment; in those diagnosed with POC tests 2.8% to 9.3% remained unwell, versus a range of 4.1% to 10.8% remaining unwell in the clinically diagnosed group (Relative risk [RR[= 0.90, 95% CI 0.69-1.17).

Prescribing outcomes were very variable with high inter-study heterogeneity (I^2=98%); in one trial in Burkina Faso (**12**) 81% of patients with negative POC test results were prescribed anti-malarial drugs. As such, in this study and two others in which there was low adherence to prescribing in line with POC test results, no significant difference in anti-malarial prescribing was found between treatment groups (Risk ratio 0.90, 95% CI 0.68-1.20). However, in the four trials in which health workers adherence to prescribing in line with POC test results was high, a large reduction in anti-malarial prescribing was found, with a risk ratio of 0.44 (95% CI 0.29-0.67).

The safety of withholding anti-malarial drugs in patients with negative POC test results has been questioned (**13**). As afore-mentioned, in high prevalence areas of malaria transmission, a negative test result might carry a high false negative rate (**4**), meaning that some patients with malaria might be missed and therefore not treated on the basis of an inaccurate POC test result. As highlighted by the practice of healthcare workers in the study by Bisoffi *et al* (**12**), a POC test result may not necessarily lead to a change in practice if the clinical suspicions of the medical practitioner are different to the POC test result. Whilst the UK has a low prevalence of malaria, faced with a very unwell febrile patient with suspected malaria and a negative POC test, one might envisage empiric anti-malarial treatment being given until the definitive laboratory microscopic analysis result is available.

A prospective study was undertaken to determine the feasibility of non-immune travellers to Kenya between June 1998 and February 1999 to self-diagnose malaria using POC tests (**14**). Patients with fever (T>38 degrees Celsius) were asked to use an HRP-2 detecting POC test (ICT Malaria Pf) with assistance only from the device's accompanying manual and no prior training. A thick blood film was also performed on each patient. Of 98 patients with fever, only 67 (68%) were able to obtain a result. Of the 11 patients that had microscopically confirmed falciparum malaria, only one was able to produce a valid test result. Of those failing to obtain a test result, 87% cited that they were unable to interpret their test result, and 71% cited that they were unable to draw sufficient fingerprick blood for analysis. This would suggest that use of POC tests should be carried out by healthcare professionals, or at least those who have had basic training in their use.

In summary, malaria POC tests have the potential to reduce inappropriate use of anti-malarials in endemic regions, bypassing the time and expertise required for microscopic analysis. POC tests may also have a role in diagnosis of placental malaria. However, due to the possibility of obtaining a false negative result, the action taken in light of a negative result is likely to depend upon the prevalence of malaria in the region of use and the beliefs held by the clinician interpreting the result. Malaria POC tests should be used by healthcare professionals or those with adequate training in their use and interpretation.

Guidelines and Recommendations:

In the WHO guidelines for the treatment of malaria, it is stipulated that prompt confirmation of malarial parasite infection using microscopy or alternatively POC tests is advised in all patients with suspected malaria, prior to initiation of anti-malarial treatment (**15**). Whilst in the UK access to

microscopic diagnostics is readily available, in parts of rural Africa POC tests could be a giant step in the direction toward making the WHO edict a reality.

The guidelines for Malaria prevention in travellers from the UK, produced by Public Health England (PHE) **(16),** state that POC tests may be useful in the hands of medical personnel accompanying an expedition to a malaria endemic region, but not for self-diagnosis by lay people. Furthermore, this guidance cautions that in the UK POC tests are not a substitute for microscopy, but they may be used alongside blood films for diagnostic purposes.

Research Questions:

1) Trials in the primary care setting to help determine whether POC tests are a viable means of ruling out malaria, and hence improve targeted referral to secondary care when appropriate, as opposed to current practice of relying upon clinical suspicion.

2) Assessment of the cost:benefit ratio of implementing use of POC tests within primary care.

Suggested next steps:

1) Studies to determine the needs in different clinical situations and settings within primary care, e.g. urgent care/out-of-hours.

2) Studies to assess the utility and feasibility of training patients travelling to rural malaria endemic regions in use of malaria POC tests.

Expected outcomes:

The use of POC tests in diagnosis of malaria would be expected to lead to faster diagnosis of malaria in suspected cases, and therefore faster initiation of treatment for those affected. Conversely, prompt acquisition of a negative test result could help reduce inappropriate prescription of anti-malarial drugs, with consequent reduction of the morbidity that can be associated with adverse drug reactions, the ever-increasing problem of drug resistance, as well as reduction of the financial burden stemming from drug wastage. A negative test result should empower the clinician to investigate alternative differentials for febrile illness.

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Appendix 1: Table of available point-of-care malaria devices

Product	Manufacturer/ Location	Blood type analysed	Sample Volume (µl)	Analysis Time	CE Mark	FDA approved	Portable	Detection Range/Limit (parasites/ µl)	Positive result outcomes	Storage Temp. (Degrees Celsius)	Method Principle	Antigen detected
Paracheck-Pf	Orchid Biomedical Systems; India	Capillary Whole Blood	5 µl	20 mins	Yes	No	Yes	Unknown	*P. falciparum*	4-45	Immunochromato-graphic Assay	PfHRP-2
ParaSight - F	Becton Dickinson; Franklin Lakes, NJ, USA	Capillary Whole blood	50 µl	Unknown	Unknown	No	Yes	>100 parasites per microliter	*P. falciparum*	Unknown	Immunochromato-graphic Assay	PfHRP-2
ICT Malaria Pf/pv	Amrad-ICT Diagnostics; Sydney, Australia	Unknown	10 µl	Unknown	Unknown	No	Yes	Unknown	*1) P. falciparum* 2) Mixed infection	2-30	Immunochromato-graphic Assay	Aldolase and PfHRP-2
ICT Malaria PF	ICT Diagnostics; New South Wales, Australia	Capillary Whole blood/venous	5 µl	15 mins	Yes	No	Yes	> 200 parasites/ µl	*P. falciparum*	4-40	Immunochromato-graphic Assay	PfHRP-2
Rapid Malaria Pf/Pv	Accu-tell; New Delhi, India	Capillary Whole blood/Venous	10 µl	15 mins	Yes	No	Yes	Unknown	*1) P. falciparum* *2) P. vivax* 3) Mixed *P.falciparum* and *P. vivax*	2-30	Immunochromato-graphic Assay	PfHRP-2 and *P.vivax* pLDH
CareStart Malaria Pf/Pan	Access Bio; New Jersey, USA	Capillary Whole blood	5 µl	20-30 mins		No	Yes	Unknown	1) *P. falciparum* malaria or mixed 2) Non-falciparum malaria	4-30	Immunochromato-graphic Assay	PfHRP-2 and Pan-pLDH
Parabank	Zephyr Biomedicals; Verna, India	Capillary Whole blood	5 µl	20 mins	Yes	No	Yes	Unknown	Pan-specific	4-30	Immunochromato-graphic Assay	Pan-pLDH

ParaHIT-F	Span Diagnostics Ltd; Surat, India	Capillary Whole blood	5 µl	15 mins	Unknown	No	Yes	>100 µl	*P. falciparum*	4-40	Immunochromato-graphic Assay	PfHRP-2
BinaxNOW Malaria Test	Alere; Maine, USA	Capillary Whole blood/venous blood	15 µl	15 minutes	Yes	Yes	Yes	>310/ µl for P.falciparum >50/ µl for non-falciparum spp	1)*P. falciparum*/ mixed 2) Non-falciparum malaria	2-37	Immunochromato-graphic Assay	PfHRP-2 and aldolase
MAKROmed Malaria Test	MACROmed manufacturing, LTD; South Africa	Capillary Whole blood	Unknown	<20 mins	Unknown	No	Unknown	>100 µl	*P. falciparum*	Unknown	Immunochromato-graphic Assay	PfHRP-2
Visitect Malaria Pf	Omega Diagnostics LTD	Capillary Whole blood/Venous blood	5 µl	15 minutes	Yes	No	Yes	Unknown	*P. falciparum*	4-40	Immunochromato-graphic Assay	PfHRP-2
Visitect Malaria Combo Pan/Pf	Omega Diagnostics LTD	Capillary Whole blood/Venous blood	5 µl	15 minutes	Yes	No	Yes	Unknown	1) *P. falciparum* or mixed 2) Non-falciparum malaria	4-30	Immunochromato-graphic Assay	Pan pLDH and PfHRP- 2
DiaMed OptiMAL-IT	BIO-RAD; California, USA	Capillary Whole blood	10 µl	20 minutes	Yes	No	Yes	>50-100/ µl	1) *P. falciparum* malaria or mixed 2) Non-falciparum malaria	2-30	Immunochromato-graphic Assay	pLDH (*P.falci-parum* specific) and pLDH (pan-specific)
OptiMAL	DiaMed AG, Cressier, Switzerland	Capillary Whole blood	Unknown	20 minutes	Yes	No	Unknown	Unknown	1) *P. falciparum* malaria or mixed 2) Non-falciparum malaria	Unknown	Immunochromato-graphic Assay	pLDH (*P.falci-parum* specific) and pLDH (pan-specific)
Malaria-Ag CELISA	Cellabs, Australia	Capillary Whole blood or	100 µl	2 hours	Yes	No	No	>5-50 / µl	*P. falciparum*	2-8	Enzyme-linked Immunosorbent Assay	PfHRP-2

		serum										
Malascan	Zephyr Biomedicals; Verna, India	Capillary Whole Blood	5 µl	20 minutes	Yes	No	Yes	Unknown	1) *P. falciparum*/ mixed 2) Non-falciparum malaria	4-30	Immunochromato-graphic Assay	PfHRP2 and aldolase
PATH Falciparum Malaria IC test	PATH; Seattle, USA	Capillary whole blood	5 µl	Unknown	Unknown	No	Yes	>100 µl	*P. falciparum*	Unknown	Unknown	PfHRP-2
Determine Malaria Pf	Abbott Laboratories; Tokyo, Japan	(Capillary Whole blood)	2 µl	30 minutes	Unknown	No	Yes	Unknown	*P. falciparum*	Unknown	Immunochromato-graphic Assay	PfHRP-2
DiaSpot Malaria	Acumen Diagnostics Inc; USA	Capillary Whole Blood	10 µl	10 minutes	Unknown	No	Yes	Unknown	*P. falciparum*	Unknown	Immunochromato-graphic Assay	PfHRP-2
Hexagon Malaria	HUMAN Diagnostics, Germany	Capillary or venous whole blood	5 µl	15 minutes	Yes	No	Yes	Unknown	1) *P. falciparum*/ mixed 2) Non-falciparum malaria	2-30	Immunochromato-graphic Assay	PfHRP2 and aldolase
SD Malaria Antigen Bioline	SD Diagnostics; Korea	Capillary Whole Blood	5 µl	15-30 minutes	Yes	No	Yes	>50/ µl	1) *P. falciparum* or mixed 2) Non-falciparum malaria an-specific	1-40	Immunochromato-graphic Assay	PfHRP-2 and pan-pLDH
Parascreen Rapid Test for Malaria Pan/Pf	Zephyr Biomedical Systems; Verna, India	Capillary Whole blood	5 µl	20 minutes	Yes	No	Yes	Unknown	1) *P. falciparum* or mixed 2) Non-falciparum malaria	4-30	Immunochromato-graphic Assay	PfHRP-2 and pan-pLDH
First Response Malaria (pLDH/HRP2 combo test)	Premier Medical Corporation; Daman, India	Whole blood	5 µl	<20 minutes	Yes	No	Yes	>200/ µl	1) *P. falciparum* or mixed 2) Non-falciparum malaria	1-40	Immunochromato-graphic Assay	PfHRP-2 and pan-pLDH

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An interactive model for the assessment of the economic costs and benefits of different rapid diagnostic tests for malaria

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Abstract

Background: Rapid diagnostic tests (RDTs) for malaria are increasingly being considered for routine use in Africa. However, many RDTs are available and selecting the ideal test for a particular setting is challenging. The appropriateness of RDT choice depends in part on patient population and epidemiological setting, and on decision makers' priorities. The model presented (available online) can be used by decision makers to evaluate alternative RDTs and assess the circumstances under which their use is justified on economic grounds.

Methods: An interactive model based on a decision-tree structure and a cost-benefit framework was designed to compare different diagnostic strategies. Variables included in the model can be modified by users, including RDT and treatment costs, test accuracies (sensitivity and specificity), probabilities for developing severe illness, case-fatality rates, and clinician response to negative test results. To illustrate how the model can be used, a comparison is made of presumptive treatment with two available RDTs, one detecting histidine-rich protein-2 (HRP2) and one detecting Plasmodium lactate dehydrogenase (pLDH). Data inputs were obtained from a study comparing the RDTs at seven sites in Uganda.

Results: Applying the model in the illustrative Ugandan context demonstrates that if only direct expenditures are considered, the pLDH test is the preferred option for adult patients except in high transmission settings, while young children are best treated presumptively in all settings. When health outcomes are considered, the HRP2 test gains an advantage in almost all settings and for all age groups. Introducing possible adverse consequences of using an antimalarial into the analysis, such as adverse drug reactions, or the development of resistance, considerably strengthens the case for using RDTs. When the model is adjusted to account for less than complete adherence to test results, the efficiency of using RDTs drops sharply.

Conclusion: Model output demonstrates that which test is preferable varies by location, depending on factors such as malaria transmission intensity and the costs and accuracies of the RDTs under consideration. Despite the uncertainties and complexities involved, adaptable models such as the one presented here can serve as a practical tool to assist policy makers in efficient deployment of new technologies.

Background

The role of RDTs and decisions in their implementation

In sub-Saharan Africa, management of febrile patients is typically characterized by over-prescription of antimalarial drugs [1-4], as clinicians often do not have access to, or do not request, laboratory testing before prescribing antimalarials [4,5]. Such practices were accepted, and even encouraged, when older, more affordable antimalarials such as chloroquine and sulphadoxine-pyrimethamine were effective. However, now that parasite resistance necessitates the introduction of new regimens such as artemisinin combination therapies (ACTs) [6-9], the strategy of presumptive treatment has become more problematic, as the new drugs are significantly more expensive and their safety profiles are not fully characterized. Use of rapid diagnostic tests (RDTs) to guide antimalarial therapy is increasingly advocated as a potentially safe and cost-effective strategy for fever case management [10-13].

With an increasingly large number of RDTs available on the market, decision-makers must consider a number of factors in determining which diagnostic test is likely to be most appropriate in a particular context. Some of these relate to qualities of the RDT itself, such as target antigen, sensitivity, specificity, shelf-life, heat sensitivity and cost. Other factors relate to the demographic and epidemiological circumstances of areas where the tests are to be deployed. Some data are available, for example from field studies of RDT accuracy in various settings, but data are lacking for other critical parameters that are likely to influence the overall costs and benefits of implementing RDTs. Even where data are available, many of these factors vary even within a single country or region, presenting a complicated picture to decision-makers.

The availability, performance and prices of diagnostic tests and treatments can vary widely over time and location, as do transmission intensity and host immunity. It is unlikely therefore that any RDT would maintain its advantage indefinitely or across all endemic areas. Similarly, economic evaluations of an RDT carried out in one setting may not be relevant in others, or lose their validity within a relatively short time as epidemiological patterns and the characteristics of competitor tests changes. For these reasons, policy makers might benefit from decision aids that incorporate available data and parameter estimates for factors that are variable, to provide up-to-date recommendations relevant to their circumstances.

Factors for consideration in choice of RDT

The presumptive treatment of fever episodes as malaria results in significant overuse of antimalarials and delays diagnosis of other illnesses [14-16]. Therefore, an important potential gain from introducing a new diagnostic test is in reducing the proportion of febrile patients who receive unnecessary antimalarial treatment. This safely reduces the cost of giving unnecessary antimalarials, and may help to avert morbidity associated with untreated non-malaria illness. An ideal RDT should therefore have high specificity to avoid false-positive results that would prompt unnecessary antimalarial treatment. At the same time, it is critical that an RDT must have high sensitivity to ensure that true cases of malaria are detected and treated appropriately.

In reality, improved sensitivity often comes at the expense of reduced specificity, and vice versa; however, it is difficult to weigh the implications of this trade-off for an individual patient or for public health, as they are often not directly comparable [17]. Mistakenly diagnosing a patient as uninfected (a false negative) may have more serious clinical consequences than mistakenly diagnosing a patient as infected (a false positive), but this will not always be true. However withholding antibiotics from a malaria test-negative individual because of an assumption the illness is due to malaria may lead to treatable bacterial disease progressing to become potentially life-threatening. Extensive overuse of antimalarials is also likely to come at a considerable cost over the longer term due to increased drug pressure leading to possible development of drug resistant parasite strains [2].

The trade-off in sensitivity and specificity is apparent in the reported accuracies of the two main classes of RDTs which currently appear most suitable for clinical use, detecting either histidine-rich protein-2 (HRP2) or Plasmodium lactate dehydrogenase (pLDH). HRP2 based assays have shown good sensitivity in a variety of field settings, and are increasingly advocated where reliable microscopy is not available [11,18]. Their potential disadvantage however, is persistence of detectable circulating antigen for up to several weeks after parasites have been eradicated [19-21], which may limit the usefulness of HRP2-based assays in areas of high malaria transmission. pLDH-based RDTs appear to be less sensitive but are more specific than HRP2 ones, as the antigen is rapidly cleared from the bloodstream [22-24]. HRP2- and pLDH-based tests also differ in the parasite species they detect: the HRP2 test detects only *Plasmodium falciparum*, while the pLDH test detects all four human malaria species.

For two main reasons, evaluations of diagnostic tests should also account for important differences in malaria epidemiology and population characteristics. Firstly, transmission intensity determines prevalence of parasitaemia and therefore, the probability of a test result being correct (the positive and negative predictive values). In many areas parasite prevalence varies through the year due to seasonal fluctuations in transmission intensity. Secondly, in high transmission areas the population

develops partial immunity with age [25]. An adult in a high transmission area, for instance, is more likely to be parasitaemic, but much less likely to develop severe malaria. A child in a low transmission area, on the other hand, is less likely to be parasitaemic but more likely to develop severe malaria once infected. The implications and benefit of using an RDT in each setting therefore differ [10,26,27].

Alongside the benefits of correct use of antimalarials, as for any medication, there are also possible negative consequences. The "harm of treatment" for an antimalarial or antibiotic includes the potential for drug toxicity, the contribution to the development of parasite (or bacterial) resistance, and the cost of the use of scarce resources [11]. Evaluations that account for these consequences can provide more comprehensive estimates of the real costs and benefits of various diagnostic strategies than those focusing only on immediate implications for management of a single fever episode.

This paper presents a model designed to incorporate local and current data and parameter estimates to assist stakeholders in identifying the most efficient tests and case management strategies. The aim was to develop a model that can be adapted to varied settings and RDTs, rather than to determine RDT cost-effectiveness in a generalized manner. The model expands on other available models, including one that compares the use of RDTs with microscopy and presumptive treatment [27], and data that demonstrate the importance of clinician response to test results [28]. The model presented here broadens the range of factors included in the analysis and also provides users with greater ability to explore policy options.

Use of the model is demonstrated here by comparing presumptive treatment with two RDTs proposed for deployment in peripheral outpatient departments in Uganda.

Methods

The model was designed to amalgamate the costs and consequences of diagnosing and treating patients according to results of either of the proposed RDTs or by presumptive treatment. The model was then populated with sample data from field studies in Uganda to illustrate its function and limitations, and to demonstrate the effect of changes in each variable on model output. While the data and output are relevant to these particular settings, they are presented here only for the purpose of illustrating use of the model, not as generalizable policy recommendations. Decision makers will want to review model parameters and modify these to their own circumstances where appropriate.

The model structure

The model is based on a decision-tree structure and cost-benefit framework, incorporating consequences of diagnosis and treatment to estimate the total costs, representing both expenditure and outcomes, for each of the tests. Strategies compared in the model include case management based on the results of two diagnostic tests, or presumptive treatment without a confirmed diagnosis.

Monetary values were assigned to consequences of diagnosis and treatment, incorporating costs of tests, medications and inpatient care, and a cost representing the value of life years lost due to incorrect diagnosis and treatment. As both costs and consequences of the different strategies are expressed in monetary terms, these are differentiated in the text by referring to either 'direct costs' to describe financial expenditures alone, or to 'total costs' where both financial expenditures and consequences in terms of value of life years lost are included. The option that incurs the lowest total cost is considered the most efficient.

Assignment of monetary values to health outcomes

The probability of death occurring was determined using estimates for the likelihood of untreated malaria and other febrile illnesses becoming severe, and subsequent case fatality rates. These were determined using expert opinion due to lack of clinical data. Different probabilities were assigned to different age groups and transmission intensities, as detailed in Table 1.

Table 1: Transition probabilities used in the model. NMFI – Non malarial febrile illness. CFR – Case fatality rate.

Probability untreated malaria becomes severe	**Age group**			**Source**
Transmission intensity	**Under 5**	**5 to 10**	**10+**	
Low	0.075	0.05	0.01	[25, 32, 41]; supplemented by expert opinion (Chris Whitty, Hugh Reyburn)
Medium	0.075	0.01	0.004	
High	0.075	0.01	0.0015	
CFR severe malaria	0.2	0.2	0.2	
Probability untreated NMFI becomes severe	0.01	0.005	0.010	
CFR NMFI	0.1	0.20	0.30	

The value assigned to a year of life lost (YLL) was initially set at $150, based on guidance from WHO for a threshold below which averting the loss of a disability adjusted life year (DALY) is considered cost-effective [29]. An alternative method used to value a YLL is to multiply per capita gross national income (GNI) by three, as discussed in a report by the WHO Commission on Macroeconomics and Health [30,31]. Results are presented for both values.

Costs were also assigned to the potential negative consequences of using antimalarials and antibiotics, or the 'harm of treatment'. The initial input used was the only current available estimate for the harm of treatment incurred by the use of antimalarials, that for every 200 treatments currently given, one life will be lost at some time in the future due to allergic reactions, development of drug resistance, use of scarce resources, and inappropriate treatment of other illnesses [11]. The baseline estimate for the harm of treatment with antibiotics was set equal to that of antimalarials. Recognizing the uncertainty around these estimates, the effects of variation in these values can also be explored by the user.

The model also accommodates the possibility that clinicians might continue to prescribe antimalarials in the face of negative test results. The values used in generating the results presented in this paper appear in Table 2. Figure 1 illustrates the possible outcomes and related costs for each diagnostic approach.

The model interface

The user interface allows for variation of input parameters, making the model adaptable to different antimalarial and RDT costs, and to different test accuracies (Figure 2). The interface also enables the user to vary estimates for key parameters with strong elements of uncertainty. These include the probability of developing severe illness by age and transmission intensity, the case fatality rates for malaria and non-malarial febrile illness, and the probability that clinicians adhere to test results.

The user can also choose the perspective of the analysis. Taking the provider financial perspective considers only direct costs of tests and treatment. Alternatively the value of years of life lost to patients due to incorrect diagnosis can be added to the analysis and varied to capture immediate health benefits for the patients. Finally, a societal

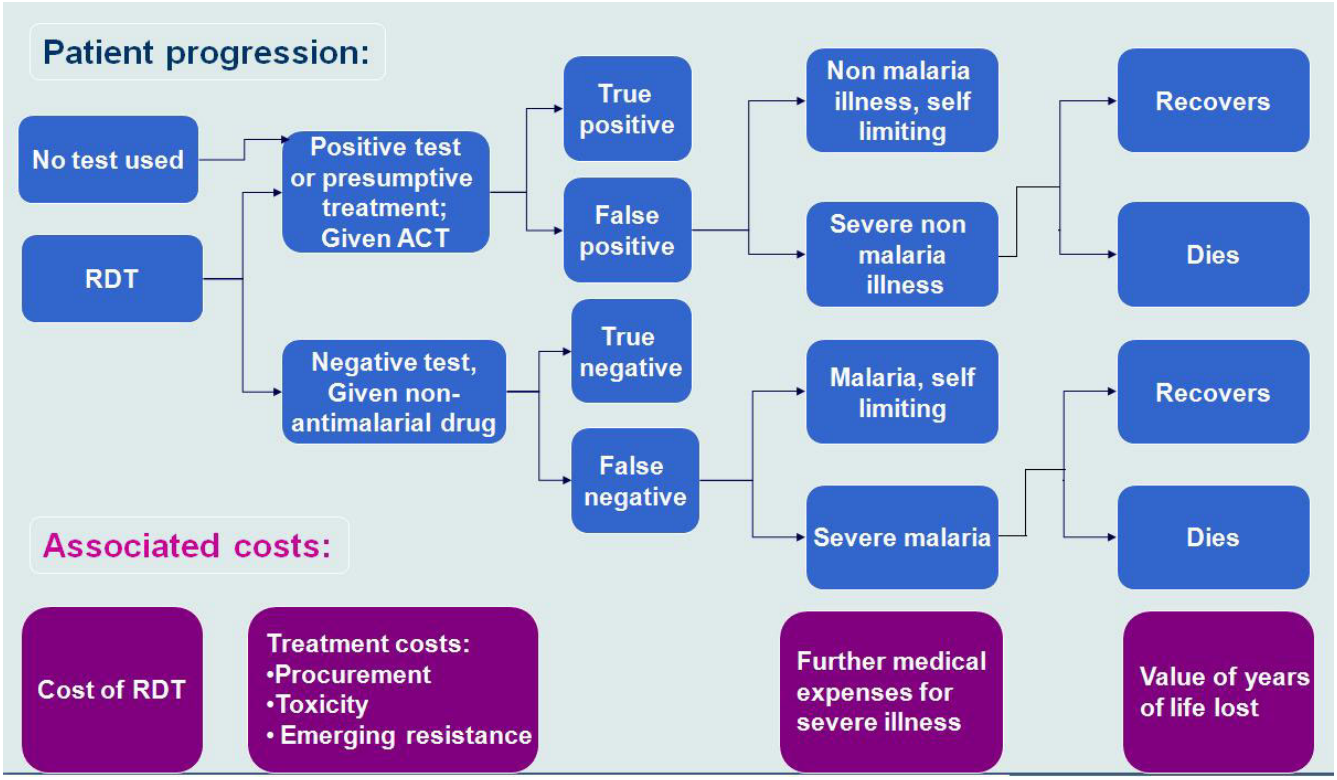

Figure 1
Decision tree structure. Patient progression and associated costs in a decision tree simulating the management of febrile patients. CFR – case fatality rate.

Table 2: Initial parameter estimates used in the model

Parameter	Base estimate and alternatives	Source
Costs:		
ACT	$1.8 (adult dose)	Uganda MoH
Antibiotic	$0.4 (adult dose)	Primary data – Joint Medical Store
pLDH RDT	$.51	Manufacturer
HRP2 RDT	$.55	Manufacturer
Harm of treatment with ACT (or antibiotic)	Every 200/2000 ACT doses currently used result in the loss of one statistical life	[11]
Inpatient care severe malaria	$12	Primary data, Kisiizi Hospital
Inpatient care severe NMFI	$20	
Accuracies:		
pLDH sensitivity	77.1%	Primary trial data
pLDH specificity	98.4%	Primary trial data
HRP2 sensitivity	98.8%	Primary trial data
HRP2 specificity	87.0%	Primary trial data
Illness progression probabilities:		
Adherence	Full adherence -100%	Variable in model
Year of Life Lost (YLL)	$150, $840	[29-31]

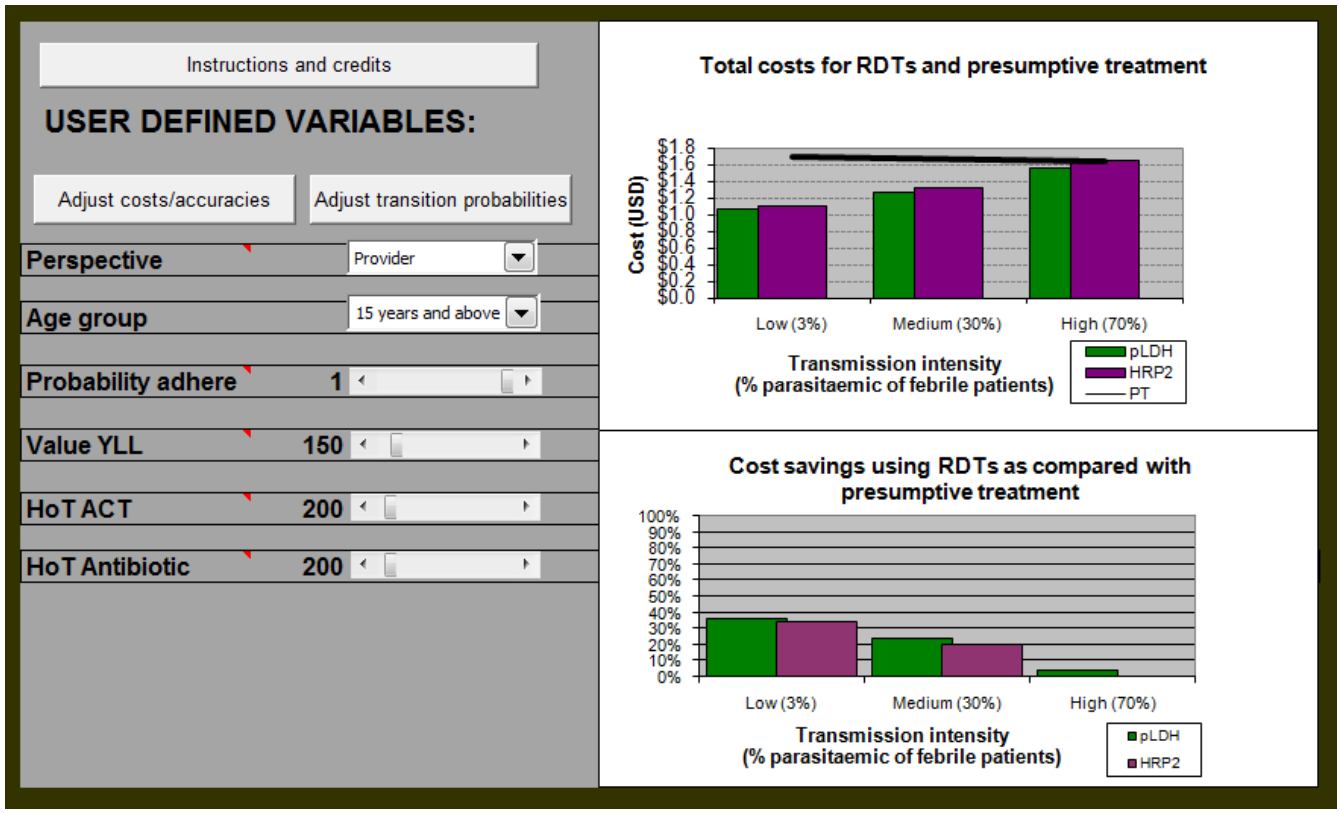

Figure 2

The user interface. RDT and drug costs, and test accuracies can be accessed and changed using the assigned button on the left hand panel, as can the probabilities of developing severe illness and case fatality rates. Other parameters can be adjusted or excluded using scroll bars on the left panel. The updated results appear on the right hand side. The bars in the top right panel show the total cost for each RDT by varying levels of prevalence, and the trendline depicts the costs for presumptive treatment. The bottom graph displays the relative cost savings for each of the RDTs using presumptive treatment as a baseline, again by prevalence level. PT – presumptive treatment

perspective can be taken, with the incorporation of the harm of treatment factor.

The model output is displayed on two graphs reflecting the difference in total costs in both absolute and relative terms, across three transmission intensities, defined by prevalence of parasitaemia amongst febrile patients [28,32]. Low transmission is characterized by a prevalence of 3% parasitaemia, 30% in medium, and 70% in high. This allows users to view the most appropriate RDT with respect to regional and seasonal variation in transmission intensity. In the top panel of Figure 2, the trendline represents the total cost in US$ of presumptive treatment in absolute terms, while each set of bars is the cost for either RDT at each transmission intensity. Where the bars fall below the trendline, use of the RDT would, therefore, be more efficient than presumptive treatment. In the lower panel the results are displayed in relative terms, using presumptive treatment as the baseline, so the bars represent the percentage by which RDTs are more efficient than presumptive treatment. Both graphs are included as in some cases the difference in relative terms might seem small, but is large in absolute terms, and vice versa.

The model was designed using Microsoft Excel® 2002 and macros were written with Microsoft Visual Basic® 6.3.

RDTs under consideration

The two RDTs evaluated for illustrative purposes in this report are Paracheck® (Orchid Biochemicals Systems Goa, India) detecting HRP2 antigens, and Parabank® (Zephyr Biomedicals, Goa, India) detecting pLDH antigens. The results are not generalisable to other settings for either the specific tests or the class of tests; these are an illustration of the uses of the model for policymakers from a particular setting. The data on RDT accuracy were obtained in clinical evaluations at sites with varied malaria epidemiology around Uganda, as has been described elsewhere [33]. Briefly, at each site, 1,000 consecutive outpatients referred to the laboratory for malaria screening, according to the usual standard of care at the health centres, were studied. For all samples where an RDT result was discordant with the microscopy result, polymerase chain reaction (PCR) was performed to confirm the presence or absence of parasitaemia. Sensitivity and specificity for each RDT were then calculated using PCR-corrected expert microscopy as the gold standard. Malaria prevalence in symptomatic patients at each site was defined as the proportion of parasitaemic patients according to the gold standard, and was assumed to be an indication of transmission intensity [34].

RDT and treatment costs

Treatment was assumed to be with artemether-lumefantrine (Coartem®), Uganda's recommended first-line treatment for uncomplicated malaria. Treatment costs for ACTs and antibiotics were determined by patient age: the cost of a dose for a child under five years of age was assumed to be one third of that for an adult, while for children aged 5 to 14 years the value used was two thirds of an adult dose. This corresponds with the figures provided by the Uganda Ministry of Health (Dr Fred Kato, Malaria Control Programme, personal communication, 27 April 2007). RDT costs were obtained from the manufacturer and incorporated an additional 15% on top of purchase price for transport and wastage [10]. Direct costs of inpatient care for patients with severe illness were estimated using primary data from Kisiizi Hospital in southwest Uganda.

Results

To demonstrate the structure and functions of the model, sample outputs are presented in a step-wise fashion, beginning with direct diagnostic and treatment costs alone. This is followed by the inclusion of patient health outcomes using the estimated values for YLL. The impact of varying levels of prescriber adherence to RDT results is then explored. The model output becomes fully comprehensive when finally the estimates of harm of treatment are incorporated. The sensitivity of the results to changes in each of the input parameters is presented as they are introduced. For simplicity, only absolute and not relative costs for each strategy are presented.

Direct cost comparison

For patients under five years of age the current cost of ACT is only marginally more expensive ($0.02) than either RDT. Therefore, if health outcomes are excluded from the analysis, presumptive treatment is the preferred option across almost all settings for this age group (Figure 3a). For patients aged five to 14 years, use of either RDT is less costly in low and medium transmission intensities, and roughly equal in the high one (result not shown). For adults both RDTs, and particularly the pLDH test, are less costly in all settings (Figure 3b).

Considering only direct expenditure excludes important factors. For example, the advantage of the pLDH test is explained in part by its lower sensitivity, resulting in fewer antimalarials being prescribed for true cases of malaria and, therefore, a lower expenditure. To capture the full cost of these untreated malaria cases in the model, the value of years of life lost due to incorrect diagnosis and treatment must be incorporated.

Introducing the value of YLL

Initially, a baseline value of $150 for a YLL was used. For patients under five years of age, the introduction of this value provides both RDTs with an advantage at the low transmission setting; at higher transmission intensities

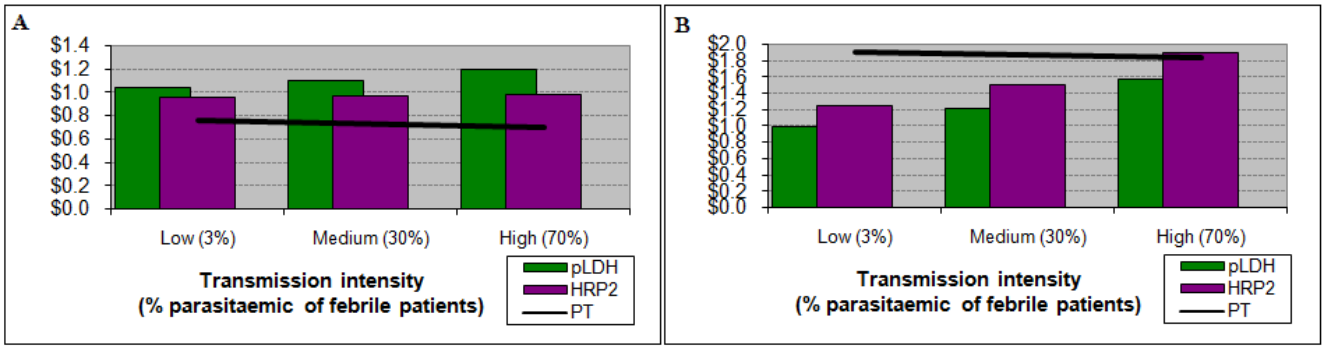

Figure 3
Results with direct costs alone. Costs of diagnosis and treatment for children under 5 (left) and for adults (right). For children the consideration of direct costs alone implies that presumptive treatment is the preferred option across all prevalences. For adults the RDT bars remain below the presumptive treatment trendline, indicating that the use of RDTs is less costly than presumptive treatment. PT – presumptive treatment

this advantage is maintained by the HRP2 test, although decreasingly so as transmission increases. Use of the pLDH test is least efficient, particularly in high-transmission areas, due primarily to its lower sensitivity and consequent failure to diagnose and treat true malaria (Figure 4a). When the value of a YLL is increased to $840 (three times Ugandan GNI per capita) [35], there is a modest further increase in the benefit of using the HRP2 test.

For older children, the HRP2 test has a small advantage over the pLDH test across all three settings, with the pLDH test being more costly than presumptive treatment at the high transmission intensity. For adults both RDTs are more efficient than presumptive treatment across all settings (Figure 4b), with substantial cost savings at sites with lower transmission.

Adherence

Results so far assume that clinicians prescribe treatment that are consistent with test results in prescribing treatments. However, consistent responses cannot be assumed given evidence from many areas showing that antimalarials are often prescribed even if test results are negative, and the degree of consistency affects comparisons [4,26]. For children aged five to 14 years for instance, the advantage gained by using the HRP2 test is lost once adherence falls below approximately 65%, and presumptive treatment becomes the preferred option.

Harm of treatment

When the harm of treatment associated with over-prescription of antimalarials is included, results change considerably in favour of either RDT. The baseline estimate

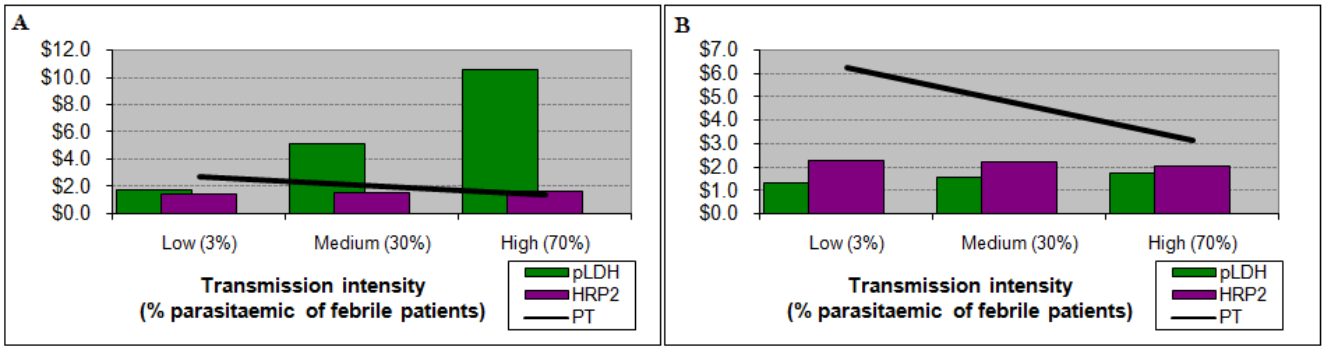

Figure 4
Results incorporating the value of health outcomes. Costs for children under 5 years (left) and adults (right), incorporating the value of life years lost. For young children presumptive treatment maintains a slight advantage over the HRP2 test, while the pLDH test would incur significantly higher costs, particularly at higher transmission intensity. For adults either test would be slightly more efficient than presumptive treatment, with a slight advantage to the HRP2 test up to very high prevalences. PT – presumptive treatment

implies that for every 200 ACTs given, one statistical life is lost in the future [11]. Figure 5a demonstrates that for children under five years, where previously presumptive treatment was the preferred option, when the harm of treatment is added to the analysis the use of RDTs is substantially more efficient across all settings. Recognizing the uncertainty around this value, a second value of 2,000 was arbitrarily chosen to observe the sensitivity of results to a lower estimate of harm of treatment. Even with much lower estimate of harm of treatment, the HRP2 test remained the most efficient choice (Figure 5b). At the medium transmission intensity, the harm of treatment value would have to be above 7,000 (i.e. prescription of 7,000 antimalarials equates to one statistical death) before presumptive treatment again becomes the more efficient option.

For older patients results are similar, with almost no difference between the two RDTs – both being 30% to 50% more efficient than presumptive treatment at the lower transmission intensities. This advantage was maintained by the HRP2 test in areas of high transmission as well. This result was robust to reduction in the value of the harm of treatment.

Discussion

Adaptable economic models as decision support tools

ACTs are a valuable resource and use of RDTs to target therapy is likely to be preferable to presumptive treatment in certain settings. A variety of RDTs are available, each with potential advantages and disadvantages, and deciding on the appropriate diagnostic approach for a given setting can be challenging. This paper presents a model which can compare different rapid diagnostic tests with one another and presumptive treatment. Policymakers can vary the parameters depending on local conditions, new data, and their own opinions where data are not available. The model demonstrates that which diagnostic strategy or test is likely to be cost-effective depends on setting, and perspective.

This model aims to be useful to stakeholders and decision makers in a number of ways. Firstly, by demonstrating the variation in performance by patient age and transmission intensity, policies may be better targeted to the local environments and patient populations. While it may not be feasible in all cases to implement policies that vary by region or population, as this may add costs and complexities to the implementation process, considering the possible variation will provide more accurate and nuanced data to inform the development of national strategies. Secondly, the interactive nature of the model allows policymakers to select which input parameters are relevant, and to use values that reflect the local settings. Thirdly, the model can be used to identify influential parameters for which values are uncertain, and to indicate the need for investing in further research to derive more accurate estimates.

Models can appear to make highly complex policy dilemmas overly simplistic, but despite all the uncertainties and complexities, decisions regarding the use of RDTs are being made, often using little more than intuitive inclination in the absence of better data. Models such as this assist in seeking to synthesise a large array of parameters that should all enter the decision making process.

Decision and policy implications in the Ugandan context

Using the RDT accuracy data available for Uganda as an illustration, the model suggests that at current RDT and ACT prices, use of the illustrative HRP2 RDT would be appropriate across most endemic settings and patient age groups. However, the results of the model depend to a great extent on whether factors such as the harm of treat-

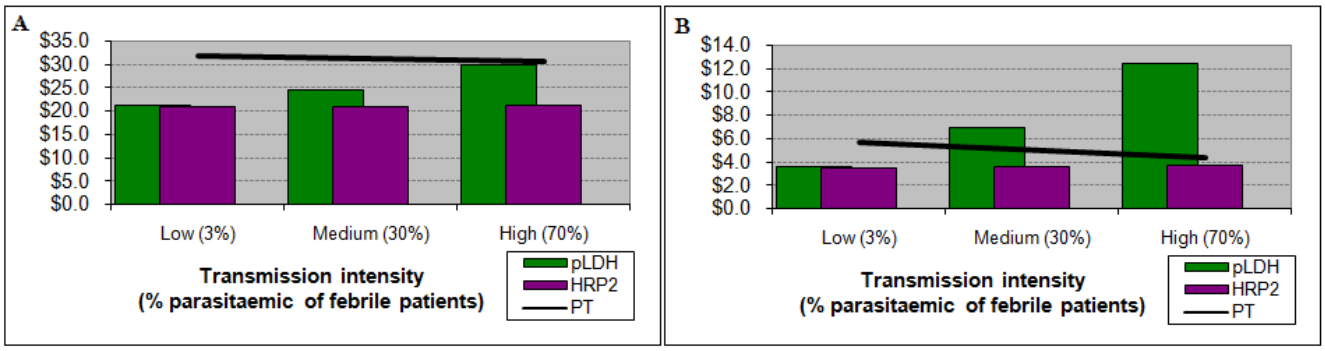

Figure 5

Results with the harm of treatment included. Total costs for children with a high (left) and low (right) estimate of harm of treatment associated with provision of antimalarials and antibiotics. Even with a very conservative estimate of the potential harm of treatment, the HRP2 test maintains an advantage across all prevalences. PT – presumptive treatment

ment and the probability of clinicians adhering to results are included in the analysis.

If the model is set to exclude the harm of treatment, as ACTs drop in price, presumptive treatment becomes justified for younger children, and the advantage of RDTs for older patients is greatly reduced. Results of the model highlight to policy makers the importance of encouraging clinicians to adhere to negative test results, if RDTs are to be an efficient use of resources.

Limitations

For some parameters in this model, such as harm of treatment, only rough estimates are available, and in many settings, local data for other parameters affecting RDT choice are unavailable. However, use of reasonable estimates and exploration of the effects of their variation in the model may provide a useful guide for decisions on RDT implementation. For purposes of illustration the model is initially populated here with current best estimates, as is the case in standard evaluations. With use of the model, users may modify these with local data where available, to tailor results as far as is possible to their own circumstances.

Two factors that were not accounted for in the model are drug efficacy, and the quality of life during illness or due to neurological sequelae. These were excluded assuming that they would have equal impact on all arms, and therefore would not alter decision recommendations. Shelf life of RDTs and stability at high temperatures are two operational factors that cannot be modelled reliably given current knowledge, but which may need to be taken into account in local settings in addition to predictive value and cost-effectiveness.

The difficulties surrounding the assignment of monetary values for years of life lost has been discussed extensively in the literature [30,36-39]. The values used in this analysis were derived from two commonly used methods – one representing a threshold for willingness to pay for a DALY averted derived by the WHO [29], and the other reflecting productivity costs by using a multiple of GNI [30,31]. In this analysis these measures have been used to value YLLs, which as opposed to DALYs do not account for a quality of life dimension. This was considered acceptable as in the context of malaria, the quality of life component is assumed to be of marginal importance in comparison to the loss of life years [40], so the two measures are almost equivalent.

The parameter surrounded with most uncertainty is the potential harm of treatment with antimalarials (or any other medication). Quantifying this requires challenging assessments such as the probability of toxicity, and the relationship of quantities of ACTs used to development of resistance, which can make the estimates appear rather arbitrary. The baseline estimate used is the only one currently available in the literature. Given this uncertainty, this parameter was varied by one order of magnitude to test its robustness, followed by a threshold analysis to determine the point where presumptive treatment again becomes more efficient.

Despite the difficulties in estimating this parameter, it is important that whatever estimates are available be accounted for in a decision model. Excluding a value for potential harm of treatment essentially can equate to saying the long term costs associated with widespread use of antibiotics or antimalarials are zero. The model allows the user to observe how changes in these values influence decision recommendations. The assignment of an equal cost to antibiotics was done on expert opinion, although users are encouraged to question this and where appropriate enter their own estimates in the model.

Conclusion

This paper presents a model that explores important parameters influencing RDT costs and benefits, that can be used by decision makers to evaluate alternative RDTs and assess the circumstances under which their use may be justified on economic grounds. It demonstrates the importance of the epidemiological setting in determining which test is most appropriate. The model is suitable for use with local data concerning test accuracies and costs of diagnostics and treatments, and allows policy makers and other stakeholders to use their own estimates for a variety of other parameters. Sample data are used to demonstrate how the model can be used to provide recommendations relevant to RDT implementation in the Ugandan context.

The question of which diagnostic approach is most cost-effective does not have a single correct answer. This paper demonstrates how in a diverse and rapidly evolving environment, adaptable and responsive models can offer guidance to encourage the most efficient deployment of new technologies.

Model availability and requirements

Project name: RDT Decision Support Model

Project home page: http://www.hefp.lshtm.ac.uk/publications

Operating system(s): All systems supporting Microsoft Office® software with Macros enabled in Excel

Programming language: Microsoft Excel ® 2002 and Microsoft Visual Basic® 6.3

Authors' contributions

YL designed the model; HH and YL wrote the initial manuscript draft; all authors contributed equally to the final content.

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CASE REPORT

Anti-JK-a Antibody in a Case of SLE Patient with *Plasmodium falciparum* Malaria Infection

Suvro Sankha Datta · Somnath Mukherjee · Prasun Bhattacharya · Krishnendu Mukherjee

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Abstract A 58 year old lady presented with high grade fever, pallor, abdominal pain, loss of appetite and swelling of legs. She was subsequently diagnosed with SLE along with infection of *Plasmodium falciparum* malaria. She was clinically pale and advised for two units of packed red cell transfusion. One of the two units was incompatible, so only one unit was issued. Subsequently, DAT and auto control were positive. Later antibody specificity was identified, which came out to be anti JK-a. Because of recent transfusion 2 weeks back, her antigenic phenotype could not be elicited. Though we could not make out whether this antibody was the result of pregnancy or transfusion induced allo anti-JK-a or SLE induced auto anti JK-a, this antibody is highly clinically significant from transfusion point of view.

Keywords SLE · DAT · Anti JK-a · Anti JK-b · Anti-Fy-a · Anti-Fy-b · Anti-Le-a · Anti-Le-b antibodies

Introduction

Unexpected antibody against Kidd blood group antigen is one of the most dangerous immune antibodies may develop in patient's serum [1]. We report a case of anti JK-a antibody in a 58 year old lady diagnosed as SLE with *falciparum* malaria infection. She also had history of recent blood transfusion. It is necessary to identify these atypical antibodies in patient's serum in order to select appropriate blood for transfusion.

Case Report

A 58 year old lady, mother of two daughters was admitted to our institute presented with high grade fever, pallor, abdominal pain, loss of appetite and swelling of legs. She also had breathing discomfort for last two weeks. Prior to her referral, she had been transfused two units of packed red cells from outside. Physical examination revealed moderate pallor, mild hepato-splenomegaly, tachypnoea (20 breaths per min), tachycardia (120 beats per min) and high grade fever (39.5 °C). On admission, her hemoglobin was 6.9 gm/dl, TLC 17,000/μl, platelet 10,000/μl. Malaria antigen test revealed *Plasmodium falciparum* positive (Parabank, Zephyr Biomedical, Goa, India) with parasitic index 0.5 %. On first day of admission her creatinine was 3.4 mg/dl, urea 133 mg/dl, total bilirubin 3.4 mg/dl, direct bilirubin 2.0 mg/dl, other parameters were normal. Though she was clinically pale and had breathing discomfort, she was advised for two units of packed red cells and four units of platelets transfusion as her platelet count was very low but no active bleeding. Her blood group was A Rh D positive. One of the two group A Rh D positive packed red cell units was incompatible in IAT phase cross match, (Diamed AG, Switzerland). So, only the compatible unit was issued to the patient. Because of the patient had also observed skin abnormalities, joint pain, fever, thrombocytopenia and pallor, an autoimmune work up was performed and revealed very high ANF 14.7 (Index value Bio Rad) (<1 negative, ≥1 positive), very high dsDNA 383.6 IU/ml (normal up to 25 IU/ml) and the patient was diagnosed as SLE with *Plasmodium falciparum* infection. Subsequently,

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direct antiglobulin test (DAT) and auto control along with antibody screening test (Diamed AG, Switzerland) were performed. Both DAT and autocontrol were positive with IgG and complement, but negative for IgM. This suggested possibility of development of IgG specific autoantibody with or without complement. Antibody screening test using three cell panel revealed positive agglutination on panel cell no. I and cell III (Table 1) (Diamed ID Diacell I, II, III Lot no. 45184.41.1 Expiry: 2009.09.28). Screening cell panel result suggested the possibility of development of anti-e, anti-Duffy (Fy-a), anti-JK-a and anti-Le-b (Lewis) antibodies. Later, anti JK-a antibody was identified on eleven cell panel (Diamed ID Diacell Lot no: 45161.03.1, expiry: 2009.09.28) (Table 2). However, whether this anti-JK-a was an autoantibody or an alloantibody, could not be elicited as the patient already had previous transfusion history two weeks back. On third day of admission, patient went into renal failure and required urgent dialysis as well as two units of packed cells. We issued again one randomly identified JK-a negative and A Rh D positive IAT phase compatible unit. No adverse events were reported during and post transfusion of that unit. On the fifth day of admission, patient's clinical condition deteriorated, she developed DIC and succumbed to *falciparum* malaria infection and sepsis.

Discussion

Kidd blood group represents ninth blood group system classified by International Society for Blood Transfusion (ISBT) [1]. Antibody against Kidd antigen are known as one of the most dangerous unexpected antibodies for causing highest prevalence and incidence rate of delayed hemolytic transfusion reaction (DHTR). The fact that unexpected antibodies to Kidd antigen, when exposed to antigens, rapidly appear in blood as an anamnestic response and then fastly disappear below detectable level may account for low detection rate [2]. The antibody, whether auto-antibody or allo-antibody, shows variability in immunoglobulin class, subclass and serological characteristics. The antibody is usually IgG or a combination of IgG and IgM; pure IgM is extremely rare. It has been generally considered that major antibody component of Kidd antibody must be IgG with complement fixing ability [3]. They are usually detected by an antiglobulin test, using a polyspecific antiglobulin or complement antisera. Although, current guidelines for pre-transfusion testing indicate that antiglobulin reagents with potent anti IgG can effectively identify such antibodies, without the need of an anti complement component [4]. Anti-JK-a as an autoantibody has been rarely described. Review of literature revealed many case reports. Furthermore, similar to our

Table 1 Screening cell panel positive in cell no. 1 and 3 and was suggestive of anti-e, anti-Fya, anti-JKa and anti-Le (Diamed ID Diacell I, II, III Lot no: 45184.41.1 EXP.2009.09.28)

Cell		Rh-hr					Kell			Duffy		Kidd		Lewis		P	MNS			LUTH		Result		
		D	C	E	c	e	K	k	Kpa	Kpb	Fya	Fyb	JKa	JKb	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	1S	37 °C	AHG
1	CCDee	R_1R_1	+	+	0	0	+	0	+	0	+	+	+	+	0	+	+	+	0	+	0	0	+	–	2+	3+
2	ccDEE	R_2R_2	+	0	0	+	0	+	+	0	+	+	0	+	+	0	+	+	+	+	+	+	+	–	–	–
3	ccddee	rr	+	0	0	+	+	+	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+	–	2+	2+

Letter [a, b] signify subtypes of red cell antigens

Table 2 Below result shows dosage phenomenon for Kidd antigen

Cell		Rh-hr					Kell			Duffy		Kidd		Lewis		P	MNS			LUTH		Result		
		D	C	E	c	e	K	k	Kpa	Kpb	Fya	Fyb	JKa	JKb	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	1S	37 °C	AHG
1	CCDee	R_1R_1	+	+	0	0	+	0	+	+	+	0	+	+	0	0	+	0	+	+	+	0	+	–	–	–
2	CCDee	R_1R_1	+	+	0	0	+	0	+	+	0	+	+	0	0	+	+	+	0	+	0	+	+	–	2+	2+
3	ccDEE	R_2R_2	+	0	+	0	0	+	0	+	+	0	0	+	+	0	+	+	0	0	+	0	+	–	–	–
4	Ccddee	r^1r	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	0	+	–	2+	2+
5	ccddEe	$r^{11}r$	0	0	+	+	0	+	0	+	+	0	0	+	+	0	+	+	+	0	+	0	+	–	–	–
6	ccddee	rr	0	0	0	+	+	+	0	+	0	+	+	0	0	+	0	0	+	0	+	0	+	–	2+	2+
7	ccddee	rr	0	0	0	+	0	+	+	+	0	+	0	+	+	0	+	+	+	+	+	0	+	–	–	–
8	ccDee	R_0r	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	+	+	+	0	0	+	–	1+	2+
9	ccddee	rr	0	0	0	+	0	+	0	+	0	+	+	+	0	0	0	0	+	+	0	0	+	–	–	–
10	ccddee	rr	0	0	0	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	–	2+	2+
11	ccddee	rr	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	0	+	0	0	+	–	–	–

Anti Kidd antibody developed in the patient showed positive agglutination in panel cell no. 2, 4, 6, 8, and 10. All these panel cells are homozygous (JKa/JKa) for Kidd antigen. Cell no. 1 and 9, though contain Kidd antigen, but in heterozygous form (JKa/JKb), thus did not show any agglutination (Diamed ID cell panel (Diacell) Lot no: 45161.03.1, Exp:28.09.09 D/O/E:28.09.2009)

Letter [a, b] signify subtypes of red cell antigens

patient, in majority of patients autoimmune diseases like SLE, autoimmune thrombocytopenia, ulcerative colitis were diagnosed. In many clinical situations where auto anti JK-a has clinical significance an underlying autoimmune disease or known antibody triggering agent is responsible for anti JK-a induced clinical relevant haemolysis. However, this patient did not have any clinically significant feature of haemolysis, she had normal LDH, and her unconjugated bilirubin was 1.4 mg/dl and did not rise thereafter.

We experienced incompatibility while cross matching red cell unit. Subsequently DAT and auto-control were positive. Antibody identification on eleven cell panel showed reactivity on panel cell no. two, four, six, eight and ten. These cell panels have Kidd antigen (JK-a) expression in homozygous (JK-a/JK-a) form and no reactivity was observed in cell panel one and nine as these cell panel have Kidd antigen expression in heterozygous form (JK-a/JK-b) (Table 2). This is very typical characteristic of Kidd antigen and known as dosage effect. So, anti-JK-a present in patient sera reacts far more strongly with JK-a/JK-a than with JK-a/JK-b cells and may be undetectable with the latter [5]. As per our work up, this patient most possibly developed warm autoantibody but we could not make out with certainty whether this anti JK-a was pregnancy or transfusion induced allo-antibody or disease induced auto-antibody. This is because her antigenic phenotyping could not be done as she had recent history of blood transfusion. Moreover, the most daunting task for the transfusion medicine specialist is the detection and identification of clinically significant allo-antibodies that may be masked by existing auto antibodies [6]. In our patient, life threatening clinical condition and time constraints did not allow us to perform adsorption and elution studies for subsequent detection of any underlying allo-antibodies.

Anti JK-a and anti JK-b are both one of the notorious unexpected antibodies causing immediate or delayed hemolytic transfusion reaction. Hence, it is mandatory for the safe transfusion that any patient with detected unexpected antibodies against Kidd blood group are supplied JK-a or JK-b antigen negative through sensitive cross matching and extended antigenic phenotyping. Auto immune hemolytic anemia (AIHA) due to anti-JK-a complement binding warm autoantibodies are extremely rare. There might be an association with auto immune diseases, as in our patient, or drug-induced antibody formation and these associations should be searched for, as they can influence the treatment. Due to the rarity of the disease, specific treatment options cannot be defined. First line treatment should therefore consist of corticosteroids like in warm AIHA. In order to prevent splenectomy in slow responders intra venous immunoglobulin (IVIG) is a valuable option [7].

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Original Article

Seroprevalence of malaria in blood donors and multi-transfused patients in Northern India: Relevance to prevention of transfusion-transmissible malaria

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Abstract

Background: Transfusion-transmissible malaria (TTM) is a major concern in malaria endemic countries. A study was therefore conducted to know sero-prevalence of malaria in blood donors and the risk of TTM to multi-transfused patients at our hospital. **Materials and Methods:** Study subjects were: eligible blood donors (n = 1000), donors deferred due to history of fever in the last 3 months (n = 100), and multi-transfused patients (n = 200). Screening for malaria was done by slide microscopy, immunochromatographic rapid diagnostic test (RDT) for malaria antigen, and anti-malaria antibody by enzyme linked immunosorbent assay. **Results:** Malaria antibody prevalence in eligible donors and donors with history of fever, thalassemia patients, and in other multi-transfused patients was 16.9%, 22%, 6%, and 15%, respectively. None of the donors were positive for malaria on microscopic examination. None of the blood donors except one donor with history of fever, tested positive with RDT. **Conclusion:** Malaria antibody prevalence in blood donors at our center is high. As blood units donated by such donors have high-risk potential, special processing may be undertaken to reduce the risk of TTM.

Keywords: Anti-malaria antibody, blood donor, multi-transfused patients, serology, transfusion-transmitted malaria

Introduction

Vector-borne malaria is a major public health problem in India; however, the malaria endemicity is quite variable across the country. [1] The annual parasite incidence (API) from our region is reported to be <2 per 1000 population whereas regions with API >5 per 1000 are scattered in the states of Rajasthan, Gujarat, Karnataka, Goa, Southern Madhya Pradesh, Chhattisgarh, Jharkhand, Orissa, and North Eastern States. [1]

In malaria endemic countries, transfusion transmitted malaria (TTM) can be a significant problem because of certain characteristics of malaria infection, i.e.,: (a) Semi-immune individuals with low level of parasitemia remain asymptomatic and can qualify as blood donors, (b) *Plasmodia*, the malarial parasite, is able to survive in blood stored at 4°C, and (c) The sensitivity of currently used methods for malaria screening (Microscopic examination: ~ 50 parasites/μL; rapid diagnostic device (RDT): ~ 100 parasites/μL) is much lower than that required to detect level of parasitemia capable of causing TTM (~ 0.00004 parasites/uL or 1-10 parasites/unit of blood).[2]

In India, strategies adopted to prevent occurrence of TTM are: a) mandatory deferral of donors with history of fever (presumably malaria) in the last 3 months and b) to test donated blood for presence of malaria infection. [3] However, prevalence of markers for malaria in blood donors and incidence of TTM in patients is scantily reported, though significant risk is highlighted by frequent case reports. [4],[5],[6],[7] Therefore, it is prudent to know the prevalence of malaria in local donor population and usefulness of currently adopted prevention strategies.

Antibodies to all four *Plasmodium species* are produced 1 to 14 days after initial infection. [3] Semi-immune malaria high-risk donors can be identified by malaria antibody screening by enzyme immunoassays (EIA), which are now available commercially. These assays provide a more sensitive and practical alternative to identify malaria high-risk donors.

A pilot study was therefore undertaken at our center to study prevalence of malaria antigen and antibody in eligible blood donors, in donors excluded on the basis of history of fever in last 3 months and in multi-transfused patients to assess the risk of TTM and usefulness of currently adopted preventive strategies.

Materials and Methods

This retrospective, cross-sectional study was conducted at the transfusion service of a tertiary care teaching hospital in the state of Uttar Pradesh in Northern India, from October 2006 to August 2008. It was approved by our Institute′s research and ethics committee. Informed consent was taken from all subjects included in the study.

Subjects and Samples

population consisted of 1000 randomly selected eligible blood donors with no history of fever in the past 3 months; 100 deferred donors due to history of suspected malaria in the past 3 months, and 200 multi-transfused patients (thalassemia patients n = 100, others n = 100) who had been transfused >10 units of packed red blood cells (PRBC) in the past 1 year. The demographic, transfusion, and other clinical details of donors and patients were recorded from blood donor cards, case files, and computer-based hospital information system.

At the time of inclusion in the study, 2 mL of blood sample in Ethylenediaminetetra-acetic acid (EDTA) vial and 5 mL of plain blood sample were collected from the subjects. EDTA sample was used for microscopic slide study and malaria antigen testing by RDT. Serum was separated from plain sample and preserved at -20°C for malaria antibody testing by enzyme linked immunosorbent assay (ELISA).

Malaria testing

examination for malaria parasite was done by thick and thin smear examination using standard methods. [8] A thick smear was drawn, stained with Giemsa stain, and observed under microscope in low power, high power, and then using oil immersion lens. If positive, a thin smear was made for species identification. In addition, all samples were also tested for malaria antigen and anti-malaria antibodies. Malaria antigen testing was done on EDTA blood samples by RDT device, which is a pan malaria test based on detection of malaria parasite-specific lactate dehydrogenase (pLDH) (PARABANK, Zephyr

Biomedicals, Goa, India) as per the manufacturer′s instructions. Results were indicated by the presence or absence of a band in the test region. Malaria antibody testing was done by commercially available malaria antibody ELISA (Pan Malaria Antibody CELISA, Cellabs Pty Ltd. Brook vale, Australia), which detects specific IgG antibody against *P. falciparum, P. vivax, P. malariae,* and *P. ovale.* Tests were done as per the manufacturer′s instructions. Samples with optical density above the cut-off value were labeled as positive.

Malaria antibody prevalence was compared among study subjects. Metselaar and van Theil criteria were used to categorize the study population on the basis of anti-malaria antibody prevalence as hypo-endemic (<10%), meso-endemic (11-50%), hyper-endemic (51-75%), and holo-endemic (>75%). [9] Correlation of antibody prevalence in blood donors in relation to gender, type of donor, frequency of donation, zone, and area of residence was also done. Malaria antibody positive and negative patients were compared with respect to age; number of PRBC transfusions received in the defined period, effect of splenectomy, or occurrence of splenomegaly to study any correlation.

Data was maintained on SPSS version 13 and Chi Square tests were applied to explore differences in antibody prevalence on the basis on donor characteristics. Student t test was used to compare the means of two variables for a single group. A *P* value of less than 0.05 was considered significant.

Results

Majority of eligible blood donors were males (93.2%), replacement donors (95.9%), urban (90.6%), residents of non-endemic zones (96.5%), and donating blood for the first time (72.5%). There were no demographic differences between the eligible and deferred blood donors. None of the eligible (n = 1000) or deferred (n = 100) blood donors were positive for malaria by slide microscopy. None of the selected donors were positive for malaria antigen by RDT; however, one of the deferred donors with recent history of fever (1%) was positive for malaria antigen by RDT. Thus, overall malaria antigen prevalence in blood donors was 0.09%. This donor was also positive for anti-malaria antibody by ELISA.

Malaria antibody prevalence in blood donors

hundred and sixty-nine (16.9%) of the eligible donors were reactive for anti-malaria antibody as compared to 22 (22%) of deferred donors with history of fever, though this difference was not statistically significant. The overall malaria antibody prevalence was 17.4%, and thus, donor population in our region was found to be meso-endemic for malaria. The demographic characteristics of blood donors and prevalence of anti-malaria antibodies are summarized in [Table - 1]. No statistically significant difference in seropositivity was evident between replacement and voluntary donors, first time and repeat donors, donors residing in non-endemic zones and those residing in endemic zones (P >0.05). However, there was significantly high prevalence of anti malaria antibody in rural donors as compared to urban donors (P = 0.001).

Malaria antibody prevalence in multi-transfused patients

antibody prevalence in thalassemia patients (6%) was much lower than in other multi-transfused patients (15%); however, the difference was not statistically significant (P >0.05). As shown in [Table - 2] there was no significant difference between mean age and number of PRBC units transfused in the last 5 years among anti-malaria antibody reactive and non-reactive thalassemia patients. A higher percentage of patients (33.3%) had been splenectomized among those reactive for anti-malaria antibodies as compared with non-reactive group (12.8%); however, the difference was not significant statistically. Among the other multi-transfused patients group also, there was no difference between the mean age, mean PRBC transfusion in the last 1 year, and presence of splenomegaly between malaria antibody non-reactive and reactive patients.

During the study period, two thalassemia patients developed malaria: one was caused by *P. falciparum* and other by *P. vivax.* Both of these patients had received PRBC transfusion two weeks prior to the malaria episode and these units were found to be malaria antibody positive on retrospective testing of donor samples. None of the other multi-transfused patients were positive for malaria by either slide microscopy or RDT.

Discussion

On the basis of overall malaria antibody prevalence (17.4%) in blood donors, our region can be categorized as meso-endemic for malaria. In a study done by Choudhry *et al.* in North Indian blood donors more than a decade ago, malaria antibody was detected in 12.39% and 19.37% of subjects by Indirect Fluorescence Antibody test (IFAT) and in-house ELISA, respectively. [10] Our results compare well with their study, as at that time the history-based donor deferral for malaria was not followed. [Table - 3] summarizes the malaria antibody prevalence in blood donors reported from various endemic and non-endemic countries and strategies adopted to prevent TTM, for comparison. As seen in [Table - 3], malaria antibody screening of blood donors is a routine method to prevent TTM in non-endemic countries. However, since malaria antibody prevalence in our donor population is high, discarding of blood on the basis of malaria antibody positive result is not a feasible option. In a study done by Oh *et al.* malaria antibody ELISA was found to have a clinical specificity of 94.0% for *P. vivax* with polymerase chain reaction (PCR) as reference method. [20] Thus, it would be prudent to evaluate and adopt additional strategies to make these units non infectious.

The statistically insignificant higher seroprevalence of malaria antibody in donors having history of fever within the last 3 months (22%) as compared with that in normal donors (16.9%) does not provide enough evidence at this stage to prove or disprove usefulness of such criteria, and results need to be confirmed on a larger sample size study to prevent unnecessary donor deferrals. Except for rural residence (33% vs. 15.2% in urban) no other donor characteristics studied, i.e., age, gender, or type of donor, had any bearing on malaria antibody prevalence. This is in concurrence with reported findings and is closely related to the agricultural practices and habits such as sleeping out of the doors and not using measures of personal protection. [21]

None of the donors was found to be positive for malaria by microscopy or RDT expect one deferred donor (0.09%) who tested positive with RDT, while in a study done by Bahadur *et al.* recently, 0.03% out of 11,736 units of donated blood were positive for malaria by RDT. [22] Therefore, blood donor screening for malaria by microscopy may not be an acceptable method as more sensitive malaria screening methods like RDT and malaria antigen testing by ELISA are now available.

Malaria antibody prevalence in multi-transfused patients was not greater than in blood donors. Therefore, no conclusion can be made as to whether malaria exposure through transfusion is a significant risk factor. Rather, the prevalence of malaria antibody in thalassemia patients (6%) was considerably lower as compared with that in donor population (17.4%), though not statistically significant. The difference could be because of lesser duration of exposure to community-acquired vector borne malaria, as majority of the thalassemia patients (90%) were less than 18 years of age whereas all the donors were above 18 years. Other studies have reported malaria incidence of 6.4% [6] and 6.9% [23] in thalassemia patients. In contrast, patients with Hb E- β Thalassemia disease at the National Thalassemia Center in Kurunegala, Sri Lanka, a region of low malarial transmission, have been found to have high frequencies of antibodies to *P. vivax* (>60%) and to a lesser degree to *P. falciparum* (>30%) from the early years of life, and the levels are significantly higher than those of age-matched controls from the same region, suggesting increased susceptibility. [24] The same study also reported significantly higher malaria antibody prevalence in thalassemics with splenomegaly or those who have undergone splenectomy. This finding was also not confirmed in our study, and the issue needs further investigation by comparing antibody prevalence in healthy non-transfused and transfused age-matched controls.

Malaria antibody prevalence in other multi-transfused group of patients in our study was 15%, which was not significantly different from the normal healthy donors acquiring malaria by vector. In comparison, in a study done by Ali *et al.* in 2004, post transfusion malaria incidence of 4.9% has been reported for multi-transfused patients. [25]

As with other transfusion-transmitted infections, suspected TTM was difficult to prove to be transmitted by transfusion, as implicated donors did not report for follow-up despite repeated requests.

In conclusion, the existing strategy of donor deferral for fever in preceding 3 months can be combined with anti-malaria antibody screening by commercially available ELISA. Anti-malaria antibody positive units may then undergo pathogen inactivation to render them non-infectious before transfusion or anti-malaria chemoprophylaxis can be given to recipients of anti-malaria antibody reactive units as targeted intervention. The ideal approach, however, would be to screen all donations for malaria by PCR which is currently the most sensitive technique (~5 parasites/ uL). [26] A recently available technique based on detection of hemozoin pigment in the neutrophils and monocytes by automated hematology cell counters should also be evaluated as it is a convenient, less costly, and objective method. [27] The usefulness of each, however, has to be evaluated in terms of TTM cases prevented and the additional costs incurred.

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The following images related to this document are available:

Photo images

[AsianJTransfusSci_2012_6_2_174_98937_b1.jpg] [AsianJTransfusSci_2012_6_2_174_98937_b2.jpg] [AsianJTransfusSci_2012_6_2_174_98937_b3.jpg]

Performance Evaluations

CE

ISO 9001: 2008
EN ISO 13485: 2012

OTHER EVALUATIONS

PARABANK®

Rapid test for the detection of Malaria (Pan)

Tulip Group

National Institute of Malaria Research (ICMR)
(Formerly Malaria Research Center)
Integrated Disease Vector Control Unit
DHS Building, Campal, Panaji, Goa- 403 001
Phone -Fax 0832-2222444 / 2421406

Ref: 4:55/797

Dated :13/12/05

M/S Zaphyr BioMedicals,
Industrial Area,
Verna, Goa

Sub; Evaluation of RDT Parabank – regarding

Dear Sirs,

As per your letters dated 22.09-2005 and 15.10.2005 regarding evaluation of rapid diagnostic test Parabank devise and dip stick, It is informed that this Institute had evaluated the said test on 100 patients. Enclosed is advance copy of preliminary report of the evaluation, which is being communicated to the NIMR headquarters at Delhi for the consideration and clearance of the Expert Committee of ICMR, New Delhi.

Final report shall be communicated to you as soon as clearance by the NIMR/ICMR is conveyed to this office.

With best wishes,

Yours sincerely,

13/12/05

(Officer-In-Charge)

Encl.: Copy of evaluation report

Officer-In Charge
Integrated Disease Vector Control Project
National Institute of Malaria Research (ICMR)
Field Station, DHS Building
Campal, Panaji - 403 001, Goa.

Evaluation of Parabank malaria diagnostic tests for (blood banks) Manufactured by M/S Zephyr Bio Medicals, Verna, Goa.

Testing Laboratory:

National Institute of Malaria Research (ICMR),

(Union Ministry of Health and Family Welfare)

Field Station, DHS Building, Campal, Panaji, Pin-403 001,

Goa, India.

Name of the Product: Parabank device and dip stick.
Parabank Dip Stick Lot No. 142004
Parabank Device Lot No. 141004

Type of Product: Immunochromatic Rapid Diagnostic kit for malaria parasites of human *Plasmodia*. Parabank test is meant for detection of Plasmodium genus specific pLDH released from parasitized RBCs containing any of the four human malaria parasite species viz., *P. vivax*, *P. falciparum*, *P. malariae* and *P. ovale*. Parabank is a test device which can detect malaria in donor's blood or stored blood to prevent malaria acquired through blood transfusion.

Principle of diagnostic tests:
1. Parabank: Immunochromatographic test capable of detecting pan malaria specific pLDH. Parabank is a qualitative, two site sandwich immunoassay, utilizing whole blood for the detection pan specific pLDH.

Period of study: Oct. to Dec. 2005

Patients enrolled: 100 routine fever cases visiting for malaria test.

Type of blood sample used: Fresh whole blood directly from finger prick of fever cases in passive collection and detection facility. Both parabank device and dipstick were tested simultaneously. Thick and thin blood smears of each patient were also simultaneously prepared for microscopy.

Time of reading of Parabank test: 15 minutes after test was performed as prescribed by the manufacturer.

13/12/05
Officer-In Charge
Integrated Disease Vector Control Project
National Institute of Malaria Research (ICMR)
Field Station, DHS Building
Campal, Panaji - 403 001, Goa

Gold Standard Used for Comparison: Blood smear stained with Giemsa stain. Blood slides blinded and read by 3 qualified Laboratory Technicians independently.

Results: Results of the testing of the Parabank device and dipstick have been summarized in Table 1 and 2 given below.

Table. 1. Comparison of malaria diagnosis with microscopy with Parabank device and Parabank dip stick diagnostic tests

Nos.	Microscopy	Parabank device	Parabank dipstick
Total Tested (% +ve)	100 (38.0)	100 (38.0)	100(38.0)
Pv (% +ve)	31 (31.0)	31(31.0)	31(31.0)
Pf (% +ve)	7 (7.0)	7 (7.0)	7(7.0)
Pv+Pf (% +ve)	0 (-)	0 (-)	0 (-)

Table: 2. Shows results of evaluation of Parabank device and Parabank dipstick malaria diagnostic tests

	P. falciparum (N=7)	*P. vivax* (N=31)	Overall
Sensitivity (%)	100	100	100
Specificity (%)	100	100	100
PPV (%)	100	100	100
NPV (%)	100	100	100
Efficacy (%)	100	100	100

PPV = Positive Predictive Value

NPV = Negative Predictive Value

Parasitaemia: In thick blood film parasites counted against 200 WBCs to work out parasitaemia / micro litre of blood taking 8000 WBCs per micro litre as standard

Parasitaemia Range :

1. *P. falciparum*: 1280 - 98800 parasites/µl of blood
2. *P. vivax* : 1280 - 22400 parasites/µl of blood

Inference: As table 2 reveals both Parabank device and Parabank dipstick diagnostic tests based on pLDH are of standard quality for the diagnosis of malaria showing absolute sensitivity, specificity and efficacy.

13/11/05

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Note: All the blood smears, parabank devices and dipsticks shall be retained with the testing laboratory for 6 months i.e. up to May 2006. These can be viewed by any party desirous of seeing and confirming results of tests or blood smears.

13/12/05

Officer-in-Charge

Officer-In Charge
Integrated Disease Vector Control Project
National Institute of Malaria Research (ICMR)
Field Station, DHS Building
Campal, Panaji - 403 001, Goa.

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संदर्भ संख्या :
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Evaluation Report of

PARABANK (Mfd. by - Zephyr Biomedicals)

'PARABANK' is a Qualitative Immunoassay Kit for Malaria (Antigen) screening at Blood Banks. It is ideally designed for blood banking purpose. Bokaro General Hospital-Blood Bank is using **'PARABANK'** as malaria screening test regularly. Its performance is highly satisfactory to us.

12/1/06

Dr. U. Mohanti

Dr. U. Mohanty
Jt. Director (M&HS)
Bokaro General Hospital

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Unit of Apollo Hospitals Enterprise Ltd.,21 Greams Lane, Off. Greams Road, Chennai - 600 006. Tel : 91-44-28293333 Fax : 91-44-28290956

TO WHOMSOEVER IT MAY CONCERN

This is to certify that the PARABANK malarial strips are being used in our Blood Bank for the past 1 year. We have also cross checked with the positives sample which were found to be good and satisfactory.

B Shyamala Sesikeran

Date : 9.8.2006

Dr. B. Shyamala Sesikeran
Sr. Blood Bank Medical Officer

Dr. B. Shyamala Sesikeran
Senior Medical Officer
Blood Bank, Apollo Hospitals
Hyderabad

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