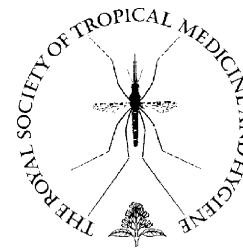




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# Assessment of three new parasite lactate dehydrogenase (pan-pLDH) tests for diagnosis of uncomplicated malaria

Carole Fogg<sup>a,b,\*</sup>, Rogers Twesigye<sup>a</sup>, Vincent Batwala<sup>c</sup>, Patrice Piola<sup>a</sup>, Carolyn Nabasumba<sup>a</sup>, James Kiguli<sup>c</sup>, Frederick Mutebi<sup>a</sup>, Christa Hook<sup>b</sup>, Martine Guillerm<sup>b</sup>, Anthony Moody<sup>b</sup>, Jean-Paul Guthmann<sup>a</sup>

<sup>a</sup> Epicentre, 8 rue Saint Sabin, 75011, Paris, France

<sup>b</sup> Médecins sans Frontières, 8 rue Saint Sabin, 75011, Paris, France

<sup>c</sup> Mbarara University of Science and Technology, P.O. Box 1410, Mbarara, Uganda

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**Summary** A study to assess the diagnostic capabilities of three parasite lactate dehydrogenase (pan-pLDH) tests, Vistapan<sup>®</sup>, Carestart<sup>™</sup> and Parabank<sup>®</sup>, was conducted in Uganda. An HRP2 test, Paracheck-Pf<sup>®</sup>, 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 ( $\chi^2$  trend,  $P < 0.001$ ); however, all tests achieved sensitivity >90% with parasitaemia  $\geq 100/\mu\text{l}$ . 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.

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## 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

\* Corresponding author. Tel.: +33 (0)1 4021 2848;

fax: +33 (0)1 4021 2803.

E-mail address: [carolefogg@yahoo.co.uk](mailto:carolefogg@yahoo.co.uk) (C. Fogg).

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/ $\mu\text{l}$ ) 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 mono-infection 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  $\geq 50\%$ ); 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 ( $\chi^2$  test, Mann-Whitney *U* test). Validity for each test was calculated overall and then stratified by age group, level of parasitaemia (parasites/ $\mu$ l 1–99,  $\geq 100$ ,  $\geq 200$ ,  $\geq 500$ ), presence/absence of fever, duration of illness (0–2 vs. 3 d and above) and a history of taking antimalarials, using comparative tests ( $\chi^2$  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* mono-infections (93.6%), *P. malariae* mono-infections (2.4%), *P. vivax* mono-infections (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  $\geq 37.5^\circ\text{C}$  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 ( $\chi^2$ )
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 ( $\chi^2$ )
Fever on presentation (axillary temp. ≥37.5°C)	99 (41.4)	31 (14.0)	130 (28.3)	<0.001 ( $\chi^2$ )
Parasitological characteristic	n = 129	n = 119	n = 248	
Asexual parasitaemia range (parasites/ $\mu$ l)	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 ( $\chi^2$ )

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/ $\mu$ l). Sensitivity was above 90% for all tests in subjects with parasitaemias ≥100 parasites/ $\mu$ l, and significantly higher than for levels of parasitaemia <100/ $\mu$ l (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 mono-infections does not permit reliable calculation of validity of non-falciparum malaria, all tests detected 100% ( $n=6$ ) of the *P. malariae* mono-infections. *Plasmodium vivax* was detected in 4/6 infections by Carestart, 2/6 by Vistapan and 1/6 by Parabank.

### 3.3. Reliability

The  $\kappa$  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 <i>n</i> <sup>a</sup>	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]

<sup>a</sup> *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* mono-infections 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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