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Research Article

Evaluation of malaria rapid diagnostic tests among children in a malaria endemic region in coastal Kenya

George O. Osanjo [a, *], Irene A. Onyango [b], Josephine Kimani [b, c], James Ochanda [b], and Julius Oyugi [d]

[a] *Department of Pharmacology and Pharmacognosy, School of Pharmacy, University of Nairobi, Kenya*
[b] *Centre for Biotechnology and Bioinformatics, University of Nairobi, Kenya*
[c] *Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*
[d] *Department of Medical Microbiology, School of Medicine, University of Nairobi, Kenya*

* **Corresponding author**: Department of Pharmacology and Pharmacognosy, School of Pharmacy, University of Nairobi, P.O. Box 19676-00202 , Nairobi, Kenya; **Tel:** +254-72-1794666; **Email**: gosanjo@uonbi.ac.ke

Background: In Kenya, malaria case management is based on clinical suspicion and detection of parasite in blood by parasitological or confirmatory diagnosis. Confirmatory diagnosis can be achieved with either microscopy or Rapid diagnostic tests (RDTs). RDTs are relatively new technologies, and their performance in actual conditions of use needs to be evaluated to provide information for appropriate use and to support decision making in procurement.

Objectives: To evaluate performance and operational characteristics of three malaria RDTs: CareStart™, First Response®, and SD Bioline™ in the field for diagnosis of infection by *Plasmodium falciparum* monospecies as well as mixed infections with *P. ovale* and *P. malariae*.

Methodology: A prospective study with blind comparisons to a gold standard was carried out at Pingilikani dispensary in Kilifi County, Kenya. Blood samples were obtained from 500 febrile children. Three RDTs: CareStart™, First Response® and SD Bioline™ were evaluated against microscopy of Giemsa stained blood films for detection of *Plasmodium falciparum* and *non-falciparum* malarial parasites. RDTs specific for *P. falciparum* only (HRP2 RDTs) and *non-falciparum* malarial parasites (HRP2/pLDH (Pf/pan) RDTs) were evaluated.

Results: *Plasmodium sp* were detected by microscopy in 242 (48.40%) study participants. *Plasmodium falciparum* species were the most prevalent (93.3%) in comparison with other *Plasmodium* species: *P. ovale* and *P. malariae* whose prevalence were 2.89% and 3.71% respectively. Compared to microscopy the sensitivities of CareStart™, SD Bioline™, and First Response® RDTs for *Plasmodium falciparum* using Pf (mono species) kits were: 95.04% (95% CI: 92.34 - 97.73), 95.04% (95% CI: 92.34 - 97.73) and 94.21% (95% CI: 91.3 - 97.11) respectively while the specificities were 78.12% (95% CI: 72.98 - 83.25), 81.10% (95% CI: 76.23 - 85.96) and 78.74% (95% CI: 73.65 - 83.82) respectively. Sensitivities of CareStart™, SD Bioline™ and First Response® RDTs for *Plasmodium falciparum* using Pf/Pan kits were: 99.02% (95% CI: 98.92 - 99.15), 99.04% (95% CI: 98.92 – 99.15) and 97.56% (95% CI: 97.78 – 97.99), respectively while the specificities were 78.46% (95% CI: 77.61 - 79.30), 78.46% (95% CI: 75.78 - 81.13) and 80.28% (95% CI: 76.73 - 83.82) respectively. CareStart™, SD Bioline™, and First Response® RDTs for *non-falciparum sp* using Pf/Pan kits both had 100% sensitivity and specificity.

Conclusion: Data from this study demonstrate that CareStart™, SD Bioline™ and First Response® RDTs have good operational characteristics and are reliable alternatives to microscopy for diagnosing malaria in children.

Key words: malaria, rapid diagnostic tests, microscopy, *Plasmodium*

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1. Introduction

Malaria remains one of the leading causes of death in tropical and sub-tropical countries, particularly in Africa where 90% of malaria cases occur in children below the age of five years (WHO, 2013). WHO recommends that treatment of malaria be initiated after the diagnosis has been confirmed parasitologically by microscopy or rapid diagnostic tests (RDTs). In many instances, however, microscopy requires technical skills and equipment that are usually unavailable or inaccessible in most malaria endemic areas. This has spurred interest in developing rapid diagnostic strategies that will be effective including in resource-limited areas where expertise in malaria diagnosis is often lacking (Reyburn et al, 2007; Bell et al, 2006).

Evaluating performance characteristics of commercially available malaria RDTs have been conducted through Panel studies (WHO, 2013). Although these studies have provided selection criteria, field evaluations are essential to obtain operational data (Murray et al, 2008; Hopkins et al, 2008; Wongsrichanalai et al, 2007).

The use of RDTs is preferable because they provide instant diagnosis and enables immediate treatment. Before rolling out large scale use of mono-*Plasmodium* species or multi-species malaria RDTs in endemic regions, choice of correct malaria RDTs should be influenced by the performance of the diagnostic strategy in the field. This study was aimed at evaluating performance characteristics of malaria rapid diagnostic tests (RDTs) for both mono and multi species in a malaria endemic region in coastal Kenya among children who are the most affected group in the population.

2. Methods

2.1 Study area

This study was done during the malaria transmission season (May - July 2014) at Pingilikani dispensary, Chonyi area in Kilifi County, Kenya. The region is classified as a moderate to high transmission area. It has been estimated that residents of this area have 22-53 infective bites per person per year (Mbogo et al, 2003). In this study area the average prevalence of parasites in children less than one year old is 13.8% while in children between 1 and 9 years of age is 40.5% (Mwangi et al, 2005).

2.2 Study design

The design was a prospective hospital based study that involved blind comparisons of test results to a gold standard.

2.3 Study population and Eligibility Criteria

All children between the ages of 2 months and 13 years, who were febrile and with suspected malaria at Pingilikani Dispensary between 25th June 2014 and 6th August 2014 were eligible for the study.

A minimal sample size of 200 patients was calculated sufficient to detect sensitivity, specificity and predictive values in patients with malaria at a two sided level of significance of 5% and 95 % level of confidence.

Both male and female children between the ages of 2 months and 13 years, who were febrile (defined as core body temperature ≥ 38.0°C or oral temperature > 37.5°C) and with suspected malaria at Pingilikani Dispensary between 25th June 2014 and 6th August 2014 were eligible for the study. Patients with severe clinical malaria symptoms such as convulsions were excluded. Patients were also excluded if consent was refused.

2.4 Blood Collection

Blood samples were obtained from febrile children, through finger pricking using Becton Dickinson (BD) contact-activated lancet, microscopy thick and thin films were prepared, and malaria RDTs testing was done. Patients with positive malaria RDTs results were treated with Artemisinin based Combination Therapy (ACT).

2.5 Assessment of rapid diagnostic test kits

The three malaria RDTs used in this study were procured from the manufacturers authorized sales representative in Kenya, that is, SAI pharmaceutical for CareStart, Value Diagnostics for SD Bioline and Intercross for First Response. All tests were performed by laboratory technologists at Pingilkani Dispensary, applying the manufacturers' instructions, and blinded as to microscopy results. HRP2 RDTs used in the study detect histidine-rich protein II expressed by *P. falciparum*. On the other hand pLDH (Pf/pan) RDTs detect *plasmodium* lactate dehydrogenase expressed by all *plasmodium* species. Consequently samples that were positive to HRP2 RDTs indicate *P. falciparum* infection. Samples that tested negative to HRP2 RDTs and positive to pLDH RDTs indicated non-falciparum infections; while those that tested positive to both HRP2 and pLDH RDTs signalled *P. falciparum* or mixed infections.

2.6 Microscopy

Thick and thin blood films were prepared for each blood sample. Thin films were prepared by smearing blood on a glass slide and fixing with methanol. The blood smears were stained with 10% Giemsa stain for 10 minutes and analyzed under the microscope at x 1,000 magnification. A minimum of 200 consecutive fields in the thick blood film were counted before the slide was classified as negative. Parasites in thick blood film were counted against 200 white blood cells (WBCs). The parasite density per ml was estimated by assuming 8,000 WBC/µl of blood (Jeremiah and Uko, 2007). Expert microscopists in the reference laboratory-Kenya Medical Research Institute/Wellcome Trust Research Programme - Kilifi (KEMRI/WTRP) were blinded to the RDT results.

2.7 Data analysis

Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) and their 95% confidence intervals (CIs) were calculated. 95% confidence intervals were calculated using the formula

P ± 1.96 √P (1-P)/N, where P is the sensitivity or specificity, and N is the number of samples from participants infected with malarial parasites (or, for specificity, from uninfected participants). Paired t test was used to calculate statistical significance at $p<0.05$.

2.8 Ethical Consideration

The study protocol was approved by Kenyatta National Hospital and University of Nairobi Ethical Review Committee (Ref: **P314/05/2014**). Written informed consent was sought from each study participant before being enrolled in the study. Participant's confidentiality was maintained. Children diagnosed with malaria were treated in accordance with the Kenya malaria treatment guidelines, that is, with artemisinin based combination therapy (ACT) for uncomplicated *P. falciparum* malaria while for non *P. falciparum* malaria (*P. vivax and P. ovale*) malaria, ACT treatment was supplemented with primaquine.

3. Results

General characteristics of the study population

A total of 500 participants of both sexes between ages of 2 months – 13years were recruited for this study between 25th June and 6th August 2014. There were more male participants (64%) compared to females (36%). Based on microscopy, majority [167 (69%)] of participants infected with malaria were children below the age of 5 years (**Table 1**).

Distribution of participants by Age and Parasite Density

Majority of participants [177 (73%)] had parasite density > 5000 Parasite / µl. Children who were 5 years old or below were more parasitaemic than those above 5 yrs of age (p=0.016) (**Table 2**).

Table 1: Disease burden in the study population

	Positive by microscopy (n/%)		Negative by microscopy (n)		
Week	**> 5years**	**2 Months - 5 years**	**> 5years**	**2 Months - 5 years**	**TOTAL**
1	25	46	9	40	120
2	20	31	15	54	120
3	8	18	5	10	41
4	15	50	20	40	125
5	7	22	29	36	94
Female	34 (14%)	63 (26%)	30	53	180 (36%)
Male	41 (17%)	104 (43%)	48	127	320 (64%)
Total	**75 (31%)**	**167 (69%)**	**78**	**180**	**500**

Table 2: Distribution of participants by Age and Parasite Density

	Parasite / µl							
Age	**≤ 100**	**101 - 200**	**201 – 500**	**501 – 1000**	**1001 - 5000**	**5001 - 50,000**	**> 50,000**	**Total**
2 Months - 5 years	1	2	6	9	21	81	46	166
> 5years	0	0	5	3	18	25	25	76
Totals	1	2	11	12	39	106	71	242

Performance characteristics of kits detecting *Plasmodium falciparum* HRP2 antigen

Compared to the reference standard, the sensitivities of SD Bioline™, First Response and . CareStart™ were 95.04 %, 94.21 % and 95.04% respectively. The specificities were 81.10%, 78.74% and 78.12% respectively. The positive predictive values of the three tests were 82.73%, 80.85% and 80.41% respectively, while the negative predictive values were 94.49 %, 93.45% and 94.33% respectively (**Table 3**).

Performance characteristics of RDTs detecting *Plasmodium* HRP2/pLDH antigens

For *Plasmodium falciparum*, the sensitivity, specificity, PPV, NPV of SD Bioline was 99.04 %, 78.46 %, 78.78% and 99.02 % respectively whereas First Response® had 97.56 % sensitivity, 80.28 % specificity, 74.07 % PPV, 98.27 % NPV. CareStart™ had sensitivity, specificity, PPV, NPV of 99.02 %, 78.46%, 78.46% and 99.02 % respectively (**Table 4**).

Table 3: Performance characteristics of three different RDTs in detecting *P. falciparum* HRP2.

	Microscopy	HRP2 (Pf)		
		CareStart™	SD Bioline™	First Response®
P. falciparum	121	143	139	142
Plasmodium species*	1	-	-	-
Mixed infection	0	0	0	0
Total Positive	122	143	139	142
Total Negative	128	107	110	108
Total No. of samples	250	250	249	249
Pf sensitivity % (95% CI)		95.04 (92.34 - 97.73)	95.04 (92.34 - 97.73)	94.21 (91.3 - 97.11)
Specificity % (95% CI)		78.12 (72.98 - 83.25)	81.10 (76.23 - 85.96)	78.74 (73.65 - 83.82)
PPV % (95% CI)		80.41 (75.48 - 85.33)	82.73 (78.03 - 87.42)	80.85 (75.96 - 85.73)
NPV (95% CI)		94.33 (91.45 - 97.20)	94.49 (91.65 - 97.32)	93.45 (90.37 - 96.52)

CI - Confidence Interval, PPV - Positive Predictive value, NPV - Negative Predictive Value
Invalid test – (1) SD Bioline, (1) First Response; **plasmodium species* ref (*P. malariae* and *P. ovale*)

Table 4: Performance characteristics of three different RDTs in detecting *Plasmodium* HRP2/pLDH antigens

	Microscopy	HRP2/pLDH (Pf/Pan)		
		Carestart™ (N)	SD Bioline™ (N)	First Response® (N)
P. falciparum	105	130	132	54
Plasmodium species*	15	15	15	7
Mixed infection	0	0	0	0
Total Positive	120	145	147	61
Total Negative	130	103	103	58
Total No. of samples	250	248	250	119
Pf sensitivity (%) (95% CI)		99.02 (98.92 - 99.15)	99.04 (98.92 – 99.15)	97.56 (97.78 – 97.99)
Pan sensitivity (%) (95% CI)		100	100	100
Total sensitivity (%)		99.51	99.52	98.78
Specificity % (95% CI)		78.46 (77.61 - 79.30)	78.46 (75.78 - 81.13)	80.28 (76.73 - 83.82)
PPV % (95% CI)		78.46 (77.61 - 79.30)	78.78 (76.14 - 81.41)	74.07 (69.41 - 78.72)
NPV (95% CI)		99.02 (98.89 - 99.14)	99.02 (98.89 - 99.14)	98.27 (97.95 - 98.58)

CI - Confidence Interval, PPV - Positive Predictive value, NPV - Negative Predictive Value
Invalid tests – (2) CareStart, (1) First Response; **P. malariae* and *P. Ovale*.

The sensitivity, specificity, PPV, NPV of all the three kits in detecting non *falciparum species* was 100%. The number of *non falciparum* species detected in this area using the Pf/Pan kits was low (15) so it may not reflect actual performance of the Pf/Pan kits. The non-falciparum malaria parasites identified were P. malariae (3.71%) and P. ovale (2.89%) (Table 4). In total Microscopic analysis showed that 226 (93.3%) blood slides were positive for *P. falciparum* while 9 were positive for *P. malariae* (3.71%) and 7 were positive for *P. ovale* (2.89%). All the three kits had sensitivities of 100% at parasite densities > 5000 Parasite / µl. However sensitivities were variable (89 - 95 %) at parasite densities <5000 Parasite /µl.

4.0 Discussion

In this study, three malaria RDTs: SD Bioline™; CareStart™ and First Response® were evaluated for their performance in diagnosis of malaria in children in comparison to Giemsa stain microscopy as a reference standard. Our findings demonstrate that SD Bioline™; CareStart™ and First Response® are reliable alternatives to Giemsa stain microscopy, with SD Bioline™ and CareStart™ HRP2 RDTs having sensitivities ≥ 95% while First Response® had a sensitivity of 94.2%. Higher sensitivity trends were observed with HRP2/pLDH (Pf/pan) RDTs (SD Bioline™, 99.04%; CareStart™, 99.02%; First Response®, 97.56%).

The findings are consistent with previous studies in other parts of the world such as central Asia (Singh et al, 2013). The results of the study also compare well with the findings of WHO product performance evaluations using cultured *P. falciparum* and frozen samples (WHO, 2013). Sensitivity can be influenced by the low numbers of the parasite. In round 5 of the WHO report (2013), First Response Pf, CareStart Pf and SD Bioline Pf had high panel detection score for the diagnosis of

Plasmodium falciparum malaria at 95%, 91% and 95%, respectively, when at least 200 parasites/ µl were present. This compares to sensitivity results obtained in this study at ≤200 parasite /µl, 100% for SD Bioline Pf, 100% CareStart Pf and 100% First Response. However the comparisons between the WHO evaluation data and those from the present study is limited by the fact that the WHO evaluation and analysis included samples with fixed parasite densities whereas the present study had variable range of parasite densities.

Recent performance evaluation of malaria RDTs has mainly focused on the detection of *Plasmodium falciparum* and other malaria species (*P. ovale, P. malariae* and *P. vivax*). In this study *falciparum* malaria detection accounted for 93.3% compared to *non-falciparum* malaria 6.7%. The *non-falciparum* malaria parasites identified were *P. malariae* (3.71%) and *P. ovale* (2.89%).

Although the three RDTs (SD Bioline, CareStart and First response) can detect malaria cases at parasitaemia levels of >200 parasites/µl, the product performances has been shown to be variable at low parasitaemia (WHO, 2011). In this study the SD Bioline; CareStart and First Response kits had sensitivities of 100% at parasite densities > 5000 parasite/µl. However, sensitivities were variable at parasite densities < 5000 parasite/µl. It is further observed that while RDTs for *P. falciparum* detected parasites at low parasite densities, the RDTs for *non-falciparum* species failed to detect parasites at low density. This indicates that confirmatory diagnosis of non-*falciparum* malaria in cases of low parasite densities should be backed up by microscopy or alternative methods.

Equally, the failure of RDTs to detect high parasite density has been observed in other studies (Palmer et al, 1998; Dyer et al, 2000). In this study there were some instances where the HRP2 RDTs failed to detect high level of parasite density (1000-5000 parasites/µl). This could be explained by parasite genetic variability. Kumar et al (2012) showed that genetic variation in *Plasmodium falciparum* HRP can be the possible cause of variability in sensitivity. Another study showed that a small number of *Plasmodium falciparum* do not express the HRP-2 due to mutations or deletions of the *hrp-2* gene leading to false negative RDT results **(**Koita et al, 2012). False negative tests have also been observed to be as a result of prozone effect - excess antibodies or antigens (Gillet et al, 2009; Luchavez et al, 2011).

Concerns over differences in performance of RDTs have previously been raised (WHO, 2011b). Performance variability has been attributed to the manufacturing process of the kits. In this study low specificity to *Plasmodium falciparum* (HRP2) observed was as a result of high number of false positives. One of the limitations of RDTs in detection of *Plasmodium falciparum* histidine-rich protein 2 (HRP-2) is the time it takes to eliminate the proteins in the blood. It has been documented that 28 days after effective treatment with antimalarial drugs, HRP-2 antigens could be detected in blood (Wongsrichanalai et al, 2007; Tijra et al, 2001).

In this study presence of false positive results was observed in some patients who had malaria therapy less than 3 weeks prior to enrolment into the study. Presence of false positives RDTs results leads to wrong diagnosis, wastage of drugs and inappropriate use of antimalarial treatments and hence the development of drug resistance. Increase in the number of false positives has also been shown to be as a result of limitation of microscopy in detecting low parasitaemia hence more accurate results would be expected if PCR was used as the reference standard (Bell et al, 2005; Hopkins et al, 2005).

One important area that needs to be considered in evaluating performance characteristics of RDTs is number of days after effective treatment that a positive RDT result can be considered to be new malaria infection. This was beyond the scope of this study and is therefore not covered in the report. There is also need to evaluate heat stability of the RDTs in the field, and the outcome of site studies would be critical in informing policy with regard to procurement.

5.0 Conclusion

Malaria RDTs performance data from this study demonstrate that CareStart™, SD Bioline™ and First Response® RDTs are reliable alternatives to microscopy for diagnosis malaria in children in malaria endemic regions such as coastal Kenya. The three RDTs can also be routinely deployed for malaria detection in the field to compliment the microscopy procedure.

Conflict of Interest declaration

The authors declare no conflict of interest.

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References

Bell D, Wongsrichanalai C and Barnwell JW (2006). Ensuring quality and access for malaria diagnosis: how can it be achieved? *Nat. Rev. Microbiol*. **4:** S7-S20.

Bell DR, Wilson DW and Martin LB (2005). False positive results of a *Plasmodium falciparum* histidine rich protein 2 detecting malaria rapid diagnostic test due to high sensitivity in a community with fluctuating low parasite density. *Am. J. Trop. Med. Hyg.* **73:**199-203.

Craig MH, Bredenkamp BL, Williams CH, Rossouw EJ, Kelly VJ, Kleinschmidt I, Martineau A and Henry GF (2002). Field and laboratory comparative evaluation of ten rapid diagnostic tests. *Trans. R. Soc. Trop. Med. Hyg*. **96:**258-265.

Dyer ME, Tjitra E, Currie BJ and Anstey NM (2000). Failure of 'pan malarial' antibody of the ICT malaria Pf/Pv immunochromatographic test to detect symptomatic *Plasmodium malariae* infection. *Trans. R. Soc. Trop. Med Hyg.* **94:**518.

Gillet P, Mori M, Van Esbroeck M, Van den Ende J and Jacobs J (2009). Assessment of the prozone effect in malaria rapid diagnostic tests. *Malar. J.* **8:** 271.

Hopkins H, Bebell L, Kambale W, Dokomajilar C, Rosenthal PJ and Dorsey G (2008). Rapid diagnostic tests for malaria at sites of varying transmission intensity in Uganda. *J. Infect. Dis.* **197:** 510-518.

Hopkins L (2011). Blood transfer devices for malaria rapid diagnostic tests: evaluation of accuracy, safety and ease of use. *Malaria J.* **10:**30.

Jeremiah ZA and Uko EK (2007). Comparative analysis of malaria parasite density using actual and assumed white blood cell counts. *Annals Trop. Paed.* **27:**75-79.

Kilian AH, Metzger WG, Mutschelknauss EJ, Kabagambe G, Langi P, Korte R and von Sonnenburg F (2000). Reliability of malaria microscopy in epidemiological studies: results of quality control. *Trop. Med. Int. Health.* **5**:3-8.

Koita OA, Doumbo OK, Ouattara A, Tall LK, Konaré A, Diakité M, Diallo M, Sagara I, Masinde GL, Doumbo SN, Dolo A, Tounkara A, Traoré I and Krogstad DJ (2012). False-negative rapid diagnostic tests for malaria and deletion of the histidine-rich repeat region of the hrp2 gene. *Am. J. Trop. Med. Hyg*. **2:**194-8.

Kumar N, Singh JP, Pande V, Mishra N, Srivastava B, Kapoor R, Valecha N and Anvikar AR (2012). Genetic variation in histidine rich proteins among Indian Plasmodium falciparum population: possible cause of variable sensitivity of malaria rapid diagnostic tests. *Malaria J.* **11:**298.

Leke RFG, Djokam RR, Mbu R, Leke RJ, Fogako J, Megnekou R, Metenou SG and Zhou Y (1999). Detection of the Plasmodium antigens histidine rich protein2 in blood of pregnant women: implications for diagnosing placental malaria. *J. Clin. Microbiol.* **37:** 2992-2996.

Luchavez J, Baker J, Alcantara S, Belizario V, Cheng Q, McCarthy JS and Bell D (2011). Laboratory demonstration of a prozone-like effect in HRP2-detecting malaria rapid diagnostic tests: implications for clinical management. *Malaria J.* **10:** 286.

Mbogo CM, Mwangangi JM, Nzovu J, Githure JI, Yan G and Beier JC (2003). Spatial and temporal heterogeneity of *Anopheles* mosquitoes and *Plasmodium falciparum* transmission along the Kenyan coast. *Am. J. Trop. Med. Hyg.* **68:**734-42.

Murray CK, Gasser RA Jr, Magill AJ and Miller RS (2008). Update on rapid diagnostic testing for malaria. *Clin. Microbiol. Rev.* **21:**97-110.

Mwangi TW, Mohammed M, Dayo H, Snow RW and Marsh K. (2005). Clinical algorithms for malaria diagnosis lack utility among people of different age groups. *Trop. Med. Int. Health.* **10:**530-536.

Palmer CJ, Lindo JF, Klaskala WI, Quesada JA, Kaminsky R, Baun MK and Ager AL (1998). Evaluation of the OptiMAL test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* malaria. *J. Clin. Microbiol.* **36:**203-206.

Reyburn H, Mbakilwa H, Mwangi R, Mwerinde O, Olomi R, Drakeley C and Whitty CJ (2007). Rapid diagnostic tests compared with malaria microscopy for guiding outpatient treatment of febrile illness in Tanzania: randomised trial. *BMJ* **334:**403.

Singh N, Bharti PK, Singh MP, Mishra S, Shukla MM, Sharma RK and Singh RK (2013). Comparative evaluation of bivalent malaria rapid diagnostic tests versus traditional methods in field with special reference to heat stability testing in central India. *PloS One.* **8:**e58080.

Tjitra E, Suprianto S, McBroom J, Currie BJ and Anstey NM (2001). Persistent ICT malaria P.f/P.v pan malarial and HRP2 antigen reactivity after treatment of *Plasmodium falciparum* malaria is associated with gametocytemia and results in false-positive diagnoses of *Plasmodium vivax* in convalescence. *J. Clin. Microbiol.* **39:** 1025-1031.

Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A and Wernsdorfer WH (2007). A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). *Am. J. Trop. Med. Hyg.* **77:** 119-127.

World Malaria Report (2009). Geneva, World Health Organization, 2009.

World Health Organization (WHO) (2011). Malaria Rapid Diagnostic test performance: result of WHO product testing of malaria RDTs round 3 (2010-2011). www.who.tdr/publications/documents/rdt3.pdf.

World Health Organization (WHO): RDT evaluation programme. www.wpro.who.int/malaria/sites/rdt/who_rdt_evaluation/2013