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# Malaria rapid diagnostic tests in endemic settings

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## Abstract

Malaria rapid diagnostic tests (RDTs) are instrument-free tests that provide results within 20 min and can be used by community health workers. RDTs detect antigens produced by the *Plasmodium* parasite such as *Plasmodium falciparum* histidine-rich protein-2 (PfHPR2) and *Plasmodium* lactate dehydrogenase (pLDH). The accuracy of RDTs for the diagnosis of uncomplicated *P. falciparum* infection is equal or superior to routine microscopy (but inferior to expert microscopy). Sensitivity for *Plasmodium vivax* is 75–100%; for *Plasmodium ovale* and *Plasmodium malariae*, diagnostic performance is poor. Design limitations of RDTs include poor sensitivity at low parasite densities, susceptibility to the prozone effect (PfHRP2-detecting RDTs), false-negative results due to PfHRP2 deficiency in the case of *pfhrp2* gene deletions (PfHRP2-detecting RDTs), cross-reactions between *Plasmodium* antigens and detection antibodies, false-positive results by other infections and susceptibility to heat and humidity. End-user's errors relate to safety, procedure (delayed reading, incorrect sample and buffer volumes) and interpretation (not recognizing invalid test results, disregarding faint test lines). Withholding antimalarial treatment in the case of negative RDT results tends to be infrequent and tendencies towards over-prescription of antibiotics have been noted. Numerous shortcomings in RDT kits' labelling, instructions for use (correctness and readability) and contents have been observed. The World Health Organization and partners actively address quality assurance of RDTs by comparative testing of RDTs, inspections of manufacturing sites, lot testing and training tools but no formal external quality assessment programme of end-user performance exists. Elimination of malaria requires RDTs with lower detection limits, for which nucleic acid amplification tests are under development.

**Keywords:** Diagnosis, endemic, end-user, histidine-rich protein-2, malaria, plasmodium, parasite lactate dehydrogenase, rapid diagnostic test

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## Malaria in the Endemic setting: Geography and Disease Burden

Malaria is endemic in 106 countries and accounted for 655 000 deaths in 2010, 86% of which occurred among children [1]. *Plasmodium falciparum* (the most lethal species) is by far the predominant species in tropical regions. *Plasmodium vivax* (tolerant to lower ambient temperatures still facilitating replication in the vector) is mainly found in South-America and Asia (where it co-occurs with *P. falciparum*), as well as in the horn of Africa [2]. *Plasmodium ovale* is mainly restricted to sub-Saharan Africa while *Plasmodium malariae* is present in most malaria endemic regions. *Plasmodium knowlesi* infections are frequently encountered in Malaysian Borneo [3] and have also been observed in other south-east Asian countries [4,5].

Infected patients develop fever and non-specific symptoms ('uncomplicated malaria'), which, in the case of *P. falciparum* infection in vulnerable patients, can quickly progress to vital organ dysfunction with coma, pulmonary oedema or shock ('severe malaria'). Severe malaria cases have also been described for *P. vivax* infections [6]. On the other hand there is asymptomatic malaria (i.e. the presence of malaria parasites without malaria symptoms), occurring in up to 90% of individuals in endemic settings [7], but also seen in low transmission areas [8,9].

Prompt and accurate diagnosis of malaria is needed to prevent progress to severe malaria. Due to the non-specific symptoms, clinical diagnosis leads to over-diagnosis and over-treatment [10–12]. Therefore, the World Health Organization (WHO) recommends parasitological confirmation of malaria before commencing treatment [13]. This recommendation, referred to as the 'Test and Treat' policy, has recently been amended with a recommendation for improved surveillance ('3T', Test, Treat, Track), including active case detection in high-risk populations in malaria elimination settings (http://www.who.int/malaria/test_treat_track/en/index.html).

## Scope of the Present Review

The present review addresses the strengths and limitations of RDTs employed for diagnosis of patients suspected of having malaria in endemic settings. It focuses on RDTs currently available to and performed by (community) health workers in remote field settings. For issues of selection, procurement and deployment of RDTs, we refer to documents issued by WHO [2] (http://www.who.int/malaria/en/) and the Foundation of Innovative Diagnostics (FIND) (http://www.finddiagnostics.org/programs/malaria-afs/).

## Malaria Rapid Diagnostic Tests: Mechanism, Target Antigens and Formats

RDTs are easy-to-use hand-held tests that provide results within 20 min. They detect *Plasmodium* antigens by an antigen-antibody reaction on a nitrocellulose strip. For *P. falciparum* detection, the target antigens are histidine-rich protein-2 (PfHPR2) and *P. falciparum*-specific *Plasmodium* lactate dehydrogenase (Pf-pLDH). The *P. vivax*-pLDH antigen is specific for *P. vivax* and pan-pLDH and aldolase are antigens common to all human *Plasmodium* species. Different RDT formats detecting different combinations of target antigens are available (Table 1, Fig. 1). For a detailed explanation of the RDT mechanism we refer to another paper in this issue (Maltha *et al.*, Malaria rapid diagnostic tests in travel medicine).

## Microscopy and Malaria RDTs in the Endemic Setting

If available, light microscopy is still the primary method of malaria diagnosis in endemic settings [14]: Giemsa-stained thick blood film analysis is cheap and enables scoring of parasite density, to identify the different *Plasmodium* species and to differentiate sexual (gametocytes) from asexual stages. However, microscopy is labour-intensive and its quality in endemic settings is often inadequate due to problems with electricity, microscopes and stains and because of shortage of competent staff [15–20].

Malaria RDTs overcome many of the limitations of microscopy. They are designed as instrument-free tests that can be used by community health workers [21]. The Test and Treat policy has fuelled deployment of RDTs (WHO, http://www.who.int/malaria/test_treat_track/en/index.html). The number of malaria RDTs used in the public sector of endemic countries has increased from <200 000 in 2005 to 88 000 000 in 2010, and an annual need for 1.5 billion RDTs has been forecasted (http://www.rollbackmalaria.org/index.html). The increased demand for malaria RDTs has led to an increased supply, with currently more than 200 RDT products and 60 manufacturers worldwide [2].

## What are the Diagnostic Characteristics of Malaria RDTs in the Endemic Setting?

A recent Cochrane meta-analysis compiled diagnostic accuracy for 74 studies assessing accuracy of RDTs for diagnosis of

**TABLE 1. Different antigens used in malaria RDTs and number of RDT products included in the list of RDT products recommended by the Global Fund to Fight AIDS, Tuberculosis and Malaria (Version6) www.theglobalfund.org/Documents/psm/PSM_QA Diagnostics_Malaria_list/**

| *Plasmodium* species targeted (number of recommended RDTs) | Antigens targeted | Two-band RDT[a] | Three-band RDT[a] |
|---|---|---|---|
| *P falciparum* ($n$ = 21) | PfHRP2 | 19 | |
| | PfHRP2 & Pf-pLDH | 1[b] | 1[c] |
| *P falciparum* and the four common human *Plasmodium* species ($n$ = 8) | PfHRP2 & pan-pLDH | | 7 |
| | Pf-pLDH & pan-pLDH | | 1 |
| | PfHRP2 & Aldolase | | 0 |
| *P falciparum* and *P vivax* ($n$ = 4) | PfHRP2 & Pv-pLDH | | 4 |
| | Pf-pLDH & Pv-pLDH | | 0[d] |
| *P vivax* ($n$ = 1) | Pv-pLDH | 1 | |

PfHRP2, *Plasmodium falciparum* histidine-rich protein 2; Pf-pLDH, *Plasmodium falciparum*-specific parasite lactate dehydrogenase; pan-pLDH, pan-*Plasmodium* pLDH; Pv-pLDH, *Plasmodium vivax*-specific pLDH.
[a]Two-band RDTs consist of a control line and a test line directed to one antigen, three-band tests contain two test lines each directed to a different target antigen.
[b]Two-band RDT targeting both PfHRP2 & Pf-pLDH at the same test line.
[c]Three-band RDT combining PfHRP2 and Pf-pLDH detection.
[d]Not available as commercial RDT format.

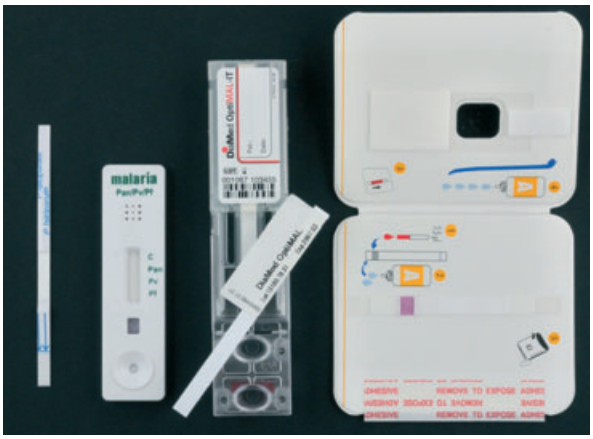


**FIG 1.** Different platforms of RDTs From left to right: dipstick (to be dipped in a tube), cassette, hybrid and cardboard. Cassettes are the easiest to use and are the most common platform (UNITAID, http://www.theglobalfund.org/en/innovativefinancing/unitaid/).

uncomplicated *P. falciparum* malaria in endemic settings [22,23]. PfHRP2-detecting RDTs displayed a slightly higher sensitivity compared with Pf-pLDH-detecting RDTs (95.0% vs. 93.2%, p = 0.34), at the trade-off of a lower specificity (95.2% vs. 98.5%, p = 0.01). The lower specificity of PfHRP2-detecting RDTs can partly be explained by the slow clearance of PfHRP2: in patients with recently treated or self-cleared infections PfHRP2 may persist in the blood for several weeks [24,25] and still be present during a new episode of fever. For *P. vivax* diagnosis, reported sensitivities for pan-pLDH, Pv-pLDH and aldolase-detecting RDTs are, respectively, 76.1–100.0% [26–37], 76.9–100.0% [33,34,38–42] and 80.0–81.4% [37,43]. A prospective field study in Peru, comparing side-to-side 11 RDT products, revealed high sensitivity (>99.0%) for 9/11 products with no difference between pan-pLDH vs. Pv-pLDH detection [33]. RDT sensitivity for *P. ovale* and *P. malariae* detection in endemic settings has hardly been assessed due to their low prevalence, but reports in travel medicine show poor results (sensitivity range 5.5–86.7% and 21.4–45.2%, respectively; see *Maltha et al.*, Malaria rapid diagnostic tests in travel medicine, in this issue). For all species, RDT sensitivity declines at lower parasite density [34,44,45].

There is much heterogeneity among reported results for RDT diagnostic accuracy, which may depend on several factors: the RDT product used [45–47], local epidemiology of malaria, the reference method used (microscopy vs. PCR), the particular end-user (e.g. experienced laboratory worker vs. community health worker) and available infrastructure.

It must be taken into account that correctly identifying the presence of malaria parasites does not imply the patient is ill due to malaria [48]. Especially in severe disease, bacterial (co-) infections may be overlooked [49–51].

Moreover, only a few studies assessed operational parameters such as proportion of invalid tests and proportion of test lines with faint intensity [33,34,52] and most field studies assessed the accuracy of RDTs *per se* rather than during routine unsupervised use. Finally, although RDT performance for the detection of *P. falciparum* in endemic settings has been shown to be equal or superior to routine microscopy [53,54], they are not as accurate as expert microscopy and do not generate all information provided by microscopy. Therefore, where established, microscopy should not be neglected for training and quality assurance.

## Limitations of Malaria RDTs Related to Design and Engineering

RDTs have inherent limitations. As mentioned above, low parasite densities (which may cause clinical disease in vulnerable patients [55]) are frequently missed. Samples with high parasite densities are vulnerable to the prozone effect (i.e. false-negative test results or test lines of low line intensity due to antigen excess). Prozone affects PfHRP2-detecting but not Pf-pLDH-detecting RDTs [52,56] and its occurrence depends on RDT product and lot [52].

False-negative results for PfHRP2-detecting RDTs have also been noted in *P. falciparum* strains lacking the *pfhrp2* gene, which may account for 25.7–41.0% of *P. falciparum* samples in the Peruvian Amazon [33,57]. A recent study from Mali retrospectively assessed more than 10-year-old filter paper samples of *P. falciparum* blood that tested negative by PfHRP2-detecting RDTs and observed negative *pfhrp2* gene amplification results [58], but in view of methodological issues these findings await confirmation. In addition, extensive diversity in the *pfhrp2* gene both within and between countries has been reported, but no correlation with RDT performance was demonstrated [59]. A recent study assessing *P. falciparum* isolates from India did, however, find differences in the detection limit of a PfHRP2-detecting RDT for different PfHRP2 antigens [60], which, however, may also be explained by variable levels of PfHRP2 production between *P. falciparum* strains [61], resulting in different PfHRP2 concentrations at identical parasite densities.

RDTs do not include wash steps, making them vulnerable to false-positive results because of non-specific bindings. Cross-reactions between *Plasmodium* species and target antigens are observed for *P. falciparum* and *P. vivax*: in a field study in Peru, up to 2.5% of *P. vivax* samples showed visible Pf-pLDH or PfHRP2 test lines for 10/12 different products tested [33]. Likewise, *P. falciparum* samples at high parasite densities may cross-react with the Pv-pLDH line (up to 29.1% of samples in 6/9 Pv-pLDH RDTs tested [62]). False-positive reactions have

been attributed to several immunological and infectious factors such as the rheumatoid factor, hepatitis C, schistosomiasis, toxoplasmosis, dengue, leishmaniasis, Chagas disease and human African Trypanosomiasis [45,63–66].

Finally, extreme temperatures and humidity affect the performance of RDTs [55,67]. Some RDT products withstand temperatures up to 40°C, but most RDTs (e.g. 32/40 (80%) products assessed in a previous study at our centre [68]) are labelled to be stable up to 30°C, which is easily exceeded in tropical settings. Pf-pLDH-detecting RDTs have been reported to be less heat resistant although some Pf-pLDH-detecting RDTs actually have extended temperature stabilities [45,67].

## Limitations of Malaria RDTs Related to End-user's Performance

Table 2 lists the most frequent errors committed by end-users in remote settings; they include critical steps related to safety and accuracy of procedure and interpretation. Reading beyond the recommended reading time proves to be a persistent error: at warm ambient temperatures, the excess sample with unbound conjugate flows back by capillary action of the sample/buffer pad and gets passively deposited on the test band, thereby generating a false-positive line, a so-called 'Backflow' phenomenon [69].

Most errors by end-users can be explained by shortcomings in packaging, labelling, correctness and readability of instructions [68,70], and unsatisfactory comprehension of 'universal' ISO15223-based graphical symbols [71]. In addition, shortcomings in other contents of RDT kits may add to poor performance, such as desiccants with absent or difficult to assess humidity indicator [72], tiny alcohol swabs [70], pipette transfer devices with no volume mark and incorrectly packaged lancets (Figs 2 and 3). A combination of (repeat) training, clear and simple bench-site job aids (in local language) and supervision visits are effectively and sustainably contributing to correct end-user's performance [21,73,74].

## Acceptability of RDTs and Adherence to RDT Test Results

The implementation of an RDT may evoke conflicting responses from the health worker (who may feel a loss of credibility when making a clinical diagnosis of malaria) and the patient (who insists on treatment irrespective of the RDT result) [70]. Further, withholding malaria treatment in the case of a negative RDT result in non-severe disease has been reported to occur infrequently in some studies [75–79] but frequently in others [80,81]. Adherence to RDT results appeared to be safe [81,82] although an improved strategy for both positive (possibility of bacterial co-infection) [50,83] and negative RDT results is needed. High frequencies of antibiotic prescription in cases of negative RDT results have been observed [80,84]. Repeat testing in the case of negative RDT results may be useful in a developing infection when antigen concentrations are still too low to be detected. For all other causes of false-negative results that are mentioned above, repeat testing is of no use. Because of antigen persistency, especially PfHRP2 as described above, RDTs cannot be used for treatment follow-up (see also 'Malaria rapid diagnostic tests in travel medicine', which can be found in this edition).

Further, although the Test and Treat policy has proven to be safe and efficacious in uncomplicated malaria [80,82,85,86], there is still debate about its universal application [87]. In particular, a positive RDT result can indicate an asymptomatic carrier who is suffering from non-malaria illness but the results may prevent the clinician from exploring other febrile diseases [88] and therefore antibiotics may be withheld from those who need them. This is of concern as bacterial infections, especially invasive salmonellosis, are observed to occur together or shortly after *P. falciparum* infections [50,89].

## How to Assure Quality of RDTs in Endemic Settings?

Substandard malaria RDTs are widespread in resource-limited settings [90,91] and lot-to-lot variations may affect performance of RDTs [92]. Regulatory approvals from high-income countries are of limited help: for instance, the requirements for the European Union's conformity label (CE Mark) in the case of malaria RDTs are purely administrative [90]. To overcome this vacuum, WHO and partners organized the 'Prequalification of Diagnostics Programme': in addition to RDT product dossier assessments, manufacturing sites are inspected for compliance with ISO13485 standards and an active post-marketing surveillance system has been installed (http://www.who.int/diagnostics_laboratory/evaluations/en/). Further, the so-called WHO/FIND Rounds assess RDTs also for diagnostic accuracy (*P. falciparum* and *P. vivax*) and heat stability (http://www.finddiagnostics.org/programs/malaria-afs/malaria/rdt_quality_control/product_testing/) and WHO/FIND further offer a lot testing programme. As to transport and storage, the development and local production of evaporative power-free cooling boxes in Cambodia and Afghanistan is of note [34,93].

At the site of performance, few tools for QC of individual RDT test kits are available. WHO/FIND produce job aids and appropriate training materials (http://www.finddiagnostics.org/

**TABLE 2. Errors committed by RDT end-users in malaria endemic settings. Study subjects were village or community health workers (Cambodia, Lao PDR, Mali, The Philippines and Zambia), staff of peripheral health centres (Malawi, Sudan and Uganda) and hospital laboratory staff (Mozambique and DR Congo). All subjects had been trained in RDT use and performance**

| Errors ranked according to chronology of test procedure | Effects/Comments | Countries | References |
|---|---|---|---|
| Not checking expiration date of the device | Confusion may arise from the way of displaying the expiry date [70] | Lao PDR, The Philippines, Uganda, Zambia | [21,70,73,74] |
| Not checking the humidity indicator of the desiccant | Humidity weakens the bonds between antibodies and nitrocellulose strip and delays particle re-solubilization [72] | Laos PDR, The Philippines, Zambia | [73,74] |
| Not using gloves | Gloves protect from blood-borne infection | Zambia | [21,73] |
| Reusing the same gloves for different patients | | Uganda, Zambia | [21,70] |
| Not identifying the cassette with the patients' name or laboratory number | Risk of inversion of results between patients | Zambia | [21,73] |
| Not cleaning/disinfecting the finger before pricking | | Lao PDR, The Philippines, Uganda, Zambia | [21,70,73,74] |
| Not allowing the finger to dry after cleaning and before pricking | Antiseptic needs enough action time | Lao PDR, The Philippines, Zambia | [21,73,74] |
| Reusing a lancet for a next patient | | Zambia | [21] |
| Desterilizing the lancet before use (by touching the bench or hands) | | Zambia | [21] |
| Pricking the wrong place on the finger (palmar instead of lateral side) | Pricking the palmar side of the finger is more painful than pricking at the side | Zambia | [21] |
| Not throwing the lancet in a sharps container | | Zambia | [21,73] |
| Dispensing the wrong volume of blood or not completely transferring the blood to the sample well (leaving blood on the wall of the well) | 1 Insufficient volume of blood may cause false-negative results<br>2 Too much blood may increase the risk or the intensity of a prozone effect [56]<br>3 Too much blood will cause decreased clearance of the strip | Malawi, Sudan, Uganda, Zambia | [21,70,73,106,107] |
| Distributing blood into the buffer well and/or buffer into the sample well | Sample and buffer well are not always unequivocally labelled [68] | Laos PDR, The Philippines | [74] |
| Substituting the buffer by another liquid (e.g. distilled water) | Use of any other liquid than the buffer provided in the RDT's kit may cause false-positive results [95] | Mali, Mozambique | [108] |
| Dispensing the wrong volume of buffer | 1 Insufficient volume of buffer will impede clearance of the strip and/or slow down migration with failure to generate a control line (invalid test results) [109]<br>2 Too high volume of buffer may cause false-positive results due to non-specific bindings | Lao PDR, Sudan, The Philippines, Uganda, Zambia | [21,70,73,74,106] |
| Not using a levelled surface to place the cassette | Decreasing of the migration time may cause false-negative results[110] | DR Congo, Lao PDR, Sudan, The Philippines, Zambia | [21,74,106] |
| Not discarding the used materials correctly | | Lao PDR, The Philippines, Zambia | [21,73,74] |
| Not respecting the correct reading time | 1 Reading too early may cause false-negative results<br>2 Reading too late may cause false-positive results due to a backflow phenomenon [111] | Lao PDR, Malawi, Sudan, The Philippines, Uganda, Zambia | [21,70,73,74,106,107] |
| Disregarding faint or weak test lines as negative | Faint test lines may be difficult to see, particularly in unfavourable light conditions (night shifts) and by elderly readers [55,70] | DR Congo[a], Lao PDR, The Philippines, Zambia | [21,73,74] |
| Not recognizing invalid test results | | DR Congo[a], Zambia | [73] |
| Interpreting line intensities as indicative of disease severity (and installing treatment accordingly) | Line intensity is not related to severity | Lao PDR, The Philippines, Uganda, | [70,74] |
| Not interpreting correctly a three- band RDT | Difficulties in defining the species involved based on the test line results | DR Congo[a], Sudan | [106] |

[a]Pierre Mukadi et al., Challenges in Malaria Research: Progress Towards Elimination, Switzerland, 10–12 October 2012.

programs/malaria-afs/) and have developed positive controls (freeze dried recombinant parasite antigen) that are currently under implementation and evaluation. Pending this, there are no controls at the bench except for cross-checking with microscopy [94]. It should further be noted that appearance of the control line is not a guarantee for correct test performance: it only testifies to correct migration of the signal antibody-antigen complex but does not control for other errors such as application of insufficient volume of sample or for use of an incorrect diluent [95].

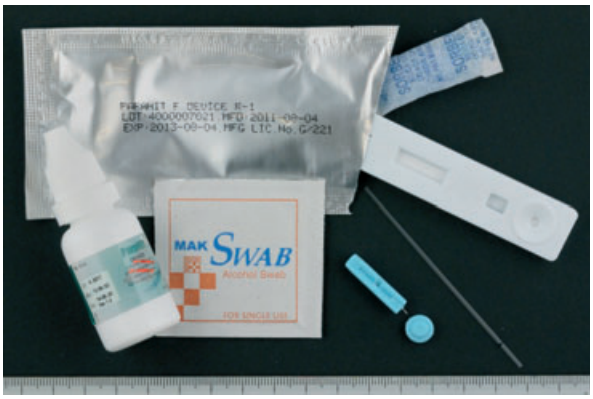


**FIG 2.** The contents of an RDT kit: sealed pouch (opened for the photo) containing cassette and desiccant, a (tiny) alcohol swab, lancet, transfer device and buffer vial. In most cases, only one buffer vial is supplied, and buffer vials are frequently lost or displaced [68]. The correct name and intended use of the RDT are not mentioned on the pouch nor on the cassette; the characters on the buffer label are very small.

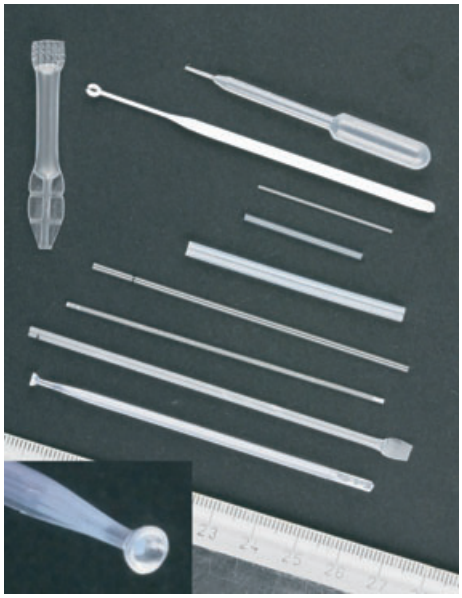

**FIG 3.** Transfer devices used to transfer capillary or venous blood to the RDT: pipettes (upper left and right), loop, capillaries, straws and inverted cup (below and insert). Some capillaries are very small, not easy to use and have no volume mark. The inverted cup proved to be the most accurate and safest blood transfer device [105].

## Special Situations and Future Directions for Malaria RDTs

*P. falciparum* malaria in pregnancy is a potentially life-threatening infection associated with miscarriage and low birth weight [96]. Infected erythrocytes attach to the placental capillaries, resulting in very low parasite densities in the peripheral blood. Microscopy fails to detect many cases of malaria infection in pregnancy [97]. As plasma PfHRP2 concentrations reflect *P. falciparum* parasite biomass [98,99], RDTs may be expected to be useful for malaria diagnosis in pregnancy. However, although PfHRP2-detecting RDTs perform better than peripheral microscopy, many cases are missed compared with PCR [100,101].

Likewise, changing trends in malaria epidemiology argue for RDTs with lower detection limits: in some areas, malaria transmission rates have declined and malaria control is shifting to the near-elimination phase. Especially in this situation it is important to also diagnose and treat asymptomatic carriers (with very low parasite densities), as they are a potential source of transmission [17,102]. Developments are focusing on nucleic acid amplification-based platforms, which have detection limits $<1$ parasite/$\mu$L, but these are still awaiting adaptation to a point-of-care format [102,103].

The decreasing burden of malaria does not imply a reduction in the numbers of RDTs used (as illness due to malaria still needs to be confirmed or excluded) but the proportion of non-malaria febrile illnesses such as dengue, respiratory tract infections, invasive salmonellosis and others is likely to increase (FIND, http://www.finddiagnostics.org/programs/malaria-afs/about-afs/). As most malaria endemic settings are resource poor, affordable diagnostic tests for such infections are highly needed. Fulfillment of this need is expected to consolidate acceptance and implementation of malaria RDTs by end-users [70,104] .

## Conclusion

Malaria RDTs are increasingly used in endemic settings and fit into the WHO 'Test and Treat' strategy. For the diagnosis of uncomplicated *P. falciparum* infection, their diagnostic sensitivity is equal or superior to routine microscopy. RDTs are subject to limitations related to design and production, as well as end-user performance, many of which can easily be prevented or remediated. Active efforts by WHO can contribute to quality assurance of RDTs along the chain of production to the bench.

## Transparency Declaration

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## References

1. World Health Organization. *World Malaria Report 2011*. 2011.
2. World Health Organization. *Good practices for selecting and procuring rapid diagnostic tests for malaria.* 2011.
3. Singh B, Kim SL, Matusop A *et al.* A large focus of naturally acquired Plasmodium knowlesi infections in human beings. *Lancet* 2004; 363: 1017–1024.
4. Kantele A, Jokiranta TS. Review of cases with the emerging fifth human malaria parasite, Plasmodium knowlesi. *Clin Infect Dis* 2011; 52: 1356–1362.
5. Antinori S, Galimberti L, Milazzo L, Corbellino M. Plasmodium knowlesi: the emerging zoonotic malaria parasite. *Acta Trop* 2012; 125: 191–201.
6. Kochar DK, Das A, Kochar SK *et al.* Severe Plasmodium vivax malaria: a report on serial cases from Bikaner in northwestern India. *Am J Trop Med Hyg* 2009; 80: 194–198.
7. Bottius E, Guanzirolli A, Trape JF, Rogier C, Konate L, Druilhe P. Malaria: even more chronic in nature than previously thought; evidence for subpatent parasitaemia detectable by the polymerase chain reaction. *Trans R Soc Trop Med Hyg* 1996; 90: 15–19.
8. Branch O, Casapia WM, Gamboa DV *et al.* Clustered local transmission and asymptomatic Plasmodium falciparum and Plasmodium vivax malaria infections in a recently emerged, hypoendemic Peruvian Amazon community. *Malar J* 2005; 4: 27.
9. Laishram DD, Sutton PL, Nanda N *et al.* The complexities of malaria disease manifestations with a focus on asymptomatic malaria. *Malar J* 2012; 11: 29.
10. Gwer S, Newton CR, Berkley JA. Over-diagnosis and co-morbidity of severe malaria in African children: a guide for clinicians. *Am J Trop Med Hyg*. 2007; 77 (Suppl 6): 6–13.
11. Chandler CI, Mwangi R, Mbakilwa H, Olomi R, Whitty CJ, Reyburn H. Malaria overdiagnosis: is patient pressure the problem? *Health Policy Plan* 2008; 23: 170–178.
12. Leslie T, Mikhail A, Mayan I *et al.* Overdiagnosis and mistreatment of malaria among febrile patients at primary healthcare level in Afghanistan: observational study. *BMJ* 2012; 345: e4389.
13. World Health Organization. *Guidelines for the treatment of malaria, second edition.* 2010.
14. World Health Organization. *Malaria Microscopy Quality Assurance Manual, version1*. 2009.
15. A research agenda for malaria eradication: diagnoses and diagnostics. *PLoS Med* 2011;8:e1000396.
16. Mukadi P, Gillet P, Lukuka A *et al.* External quality assessment of malaria microscopy in the Democratic Republic of the Congo. *Malar J* 2011; 10: 308.
17. Alonso PL, Barnwell JW, Bell D *et al.* A research agenda for malaria eradication: diagnoses and diagnostics. *PLoS Med* 2011; 8: e1000396.
18. Maguire JD, Lederman ER, Barcus MJ *et al.* Production and validation of durable, high quality standardized malaria microscopy slides for teaching, testing and quality assurance during an era of declining diagnostic proficiency. *Malar J* 2006; 5: 92.
19. McKenzie FE, Sirichaisinthop J, Miller RS, Gasser RA Jr, Wongsrichanalai C. Dependence of malaria detection and species diagnosis by microscopy on parasite density. *Am J Trop Med Hyg* 2003; 69: 372–376.
20. McMorrow ML, Masanja MI, Abdulla SM, Kahigwa E, Kachur SP. Challenges in routine implementation and quality control of rapid diagnostic tests for malaria–Rufiji District, Tanzania. *Am J Trop Med Hyg* 2008; 79: 385–390.
21. Counihan H, Harvey SA, Sekeseke-Chinyama M *et al.* Community health workers use malaria rapid diagnostic tests (RDTs) safely and accurately: results of a longitudinal study in Zambia. *Am J Trop Med Hyg* 2012; 87: 57–63.
22. Abba K, Deeks JJ, Olliaro P *et al.* Rapid diagnostic tests for diagnosing uncomplicated P falciparum malaria in endemic countries. *Cochrane Database Syst Rev* 2011; 6: CD008122
23. Young T, Abba K, Deeks JJ *et al.* Cochrane Column * Rapid diagnostic tests can extend access of diagnostic services for uncomplicated Plasmodium falciparum malaria * Commentary: Rapid diagnostic tests for diagnosing uncomplicated Plasmodium falciparum malaria in endemic countries (Review) * Approach to conducting Cochrane Diagnostic Test Accuracy Reviews. *Int J Epidemiol* 2012; 41: 607–610.
24. Iqbal J, Siddique A, Jameel M, Hira PR. Persistent histidine-rich protein 2, parasite lactate dehydrogenase, and panmalarial antigen reactivity after clearance of Plasmodium falciparum monoinfection. *J Clin Microbiol* 2004; 42: 4237–4241.
25. Mayxay M, Pukrittayakamee S, Chotivanich K, Looareesuwan S, White NJ. Persistence of Plasmodium falciparum HRP-2 in successfully treated acute falciparum malaria. *Trans R Soc Trop Med Hyg* 2001; 95: 179–182
26. Andrade BB, Reis-Filho A, Barros AM *et al.* Towards a precise test for malaria diagnosis in the Brazilian Amazon: comparison among field microscopy, a rapid diagnostic test, nested PCR, and a computational expert system based on artificial neural networks. *Malar J* 2010; 9: 117.
27. Arrospide N, Flores R, Ruiz J. Evaluación de una prueba rápida para el diagnostico de Malaria en áreas endémicas del Perú. *Rev perú med exp salud publica* 2006; 23: 81–86.
28. Ashley EA, Touabi M, Ahrer M *et al.* Evaluation of three parasite lactate dehydrogenase-based rapid diagnostic tests for the diagnosis of falciparum and vivax malaria. *Malar J* 2009; 8: 241
29. Bendezu J, Rosas A, Grande T *et al.* Field evaluation of a rapid diagnostic test (Parascreen) for malaria diagnosis in the Peruvian Amazon. *Malar J* 2010; 9: 154
30. Bharti PK, Silawat N, Singh PP *et al.* The usefulness of a new rapid diagnostic test, the First Response Malaria Combo (pLDH/HRP2) card test, for malaria diagnosis in the forested belt of central India. *Malar J* 2008; 7: 126
31. Ferro BE, Gonzalez IJ, Carvajal F, Palma GI, Saravia NG. Performance of OptiMAL(R) in the diagnosis of Plasmodium vivax and Plasmodium falciparum infections in a malaria referral center in Colombia. *Mem Inst Oswaldo Cruz* 2002; 97: 731–735.
32. Malik S, Khan S, Das A, Samantaray JC. Plasmodium lactate dehydrogenase assay to detect malarial parasites. *Natl Med J India* 2004; 17: 237–239.
33. Maltha J, Gamboa D, Bendezu J *et al.* Rapid Diagnostic Tests for Malaria Diagnosis in the Peruvian Amazon: impact of pfhrp2 Gene Deletions and Cross-Reactions. *PLoS ONE* 2012; 7: e43094
34. Mikhail AF, Leslie TJ, Mayan MI *et al.* Field trial of three different Plasmodium vivax-detecting rapid diagnostic tests with and without evaporative cool box storage in Afghanistan. *Malar J* 2011; 10: 169

35. Moges B, Amare B, Belyhun Y *et al.* Comparison of CareStart HRP2/pLDH COMBO rapid malaria test with light microscopy in north-west Ethiopia. *Malar J* 2012; 11: 234
36. Tarazona AS, Zerpa LS, Mendoza Requena D, Llanos-Cuentas A, Magill A. Evaluation of the rapid diagnostic test OptiMAL for diagnosis of malaria due to Plasmodium vivax. *Braz J Infect Dis* 2004; 8: 151–155.
37. van den Broek I, Hill O, Gordillo F *et al.* Evaluation of three rapid tests for diagnosis of P falciparum and P vivax malaria in Colombia. *Am J Trop Med Hyg* 2006; 75: 1209–1215.
38. Alam MS, Mohon AN, Mustafa S *et al.* Real-time PCR assay and rapid diagnostic tests for the diagnosis of clinically suspected malaria patients in Bangladesh. *Malar J* 2011; 10: 175
39. Kim SH, Nam MH, Roh KH *et al.* Evaluation of a rapid diagnostic test specific for Plasmodium vivax. *Trop Med Int Health* 2008; 13: 1495–1500.
40. Lee SW, Jeon K, Jeon BR, Park I. Rapid diagnosis of vivax malaria by the SD Bioline Malaria Antigen test when thrombocytopenia is present. *J Clin Microbiol* 2008; 46: 939–942.
41. Meena M, Joshi D, Joshi R *et al.* Accuracy of a multispecies rapid diagnostic test kit for detection of malarial parasite at the point of care in a low endemicity region. *Trans R Soc Trop Med Hyg* 2009; 103: 1237–1244.
42. Sharew B, Legesse M, Animut A, Jima D, Medhin G, Erko B. Evaluation of the performance of CareStart Malaria Pf/Pv Combo and Paracheck Pf tests for the diagnosis of malaria in Wondo Genet, southern Ethiopia. *Acta Trop* 2009; 111: 321–324.
43. Pabón A, Bader G, Yánez J *et al.* Evaluación de la prueba rápida inmunocromatográfica Binax NOW-« ICT Pf/Pv para el diagnostico del paludismo en un área endémica de Colombia. *Biomédica* 2007; 27: 225–235.
44. Endeshaw T, Graves PM, Ayele B *et al.* Performance of local light microscopy and the ParaScreen Pan/Pf rapid diagnostic test to detect malaria in health centers in Northwest Ethiopia. *PLoS ONE* 2012; 7: e33014
45. World Health Organization *Malaria Rapid Diagnostic Test Performance; Results of WHO product testing of malaria RDTs: Round 3 (*2011) 2011.
46. World Health Organization *Malaria Rapid Diagnostic Test Performance; Results of WHO product testing of malaria RDTs: Round 2 (*2009) 2010.
47. World Health Organization *Malaria Rapid Diagnostic Test Performance; Results of WHO product testing of malaria RDTs: Round 1 (*2008) 2009
48. Bisoffi Z, Sirima SB, Menten J *et al.* Accuracy of a rapid diagnostic test on the diagnosis of malaria infection and of malaria-attributable fever during low and high transmission season in Burkina Faso. *Malar J* 2010; 9: 192
49. Maltha J, Jacobs J. Iron deficiency and malaria mortality: possible implication of invasive bacterial diseases. *Clin Infect Dis* 2012; 55: 748
50. Mtove G, Amos B, von SL *et al.* Invasive salmonellosis among children admitted to a rural Tanzanian hospital and a comparison with previous studies. *PLoS ONE* 2010; 5: e9244
51. Phoba MF, Lunguya O, Mayimon DV *et al.* Multidrug-resistant Salmonella enterica, Democratic Republic of the Congo. *Emerg Infect Dis* 2012; 18: 1692–1694.
52. Gillet P, Scheirlinck A, Stokx J *et al.* Prozone in malaria rapid diagnostics tests: how many cases are missed?. *Malar J* 2011; 10: 166
53. Batwala V, Magnussen P, Nuwaha F. Are rapid diagnostic tests more accurate in diagnosis of plasmodium falciparum malaria compared to microscopy at rural health centres?. *Malar J* 2010; 9: 349
54. de Oliveira AM, Skarbinski J, Ouma PO *et al.* Performance of malaria rapid diagnostic tests as part of routine malaria case management in Kenya. *Am J Trop Med Hyg* 2009; 80: 470–474.
55. Murray CK, Gasser RA, Jr, Magill AJ, Miller RS. Update on Rapid Diagnostic Testing for Malaria. *Clin Microbiol Rev* 2008; 21: 97–110.
56. Gillet P, Mori M, Van Esbroeck M, Van Den Ende J, Jacobs J. Assessment of the prozone effect in malaria rapid diagnostic tests. *Malar J* 2009; 30: 8
57. Gamboa D, Ho MF, Bendezu J *et al.* A large proportion of P falciparum isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. *PLoS ONE* 2010; 5: e8091
58. Koita OA, Doumbo OK, Ouattara A *et al.* False-negative rapid diagnostic tests for malaria and deletion of the histidine-rich repeat region of the hrp2 gene. *Am J Trop Med Hyg* 2012; 86: 194–198.
59. Baker J, Ho MF, Pelecanos A *et al.* Global sequence variation in the histidine-rich proteins 2 and 3 of Plasmodium falciparum: implications for the performance of malaria rapid diagnostic tests. *Malar J* 2010; 9: 129
60. Kumar A, Chery L, Biswas C *et al.* Malaria in South Asia: prevalence and control. *Acta Trop* 2012; 121: 246–255.
61. Baker J, Gatton ML, Peters J, Ho MF, McCarthy JS, Cheng Q. Transcription and expression of Plasmodium falciparum histidine-rich proteins in different stages and strains: implications for rapid diagnostic tests. *PLoS ONE* 2011; 6: e22593
62. Maltha J, Gillet P, Cnops L, Van Den Ende J, Van Esbroeck M, Jacobs J. Malaria rapid diagnostic tests: plasmodium falciparum infections with high parasite densities may generate false positive Plasmodium vivax pLDH lines. *Malar J* 2010; 9: 198
63. Leshem E, Keller N, Guthman D *et al.* False-positive Plasmodium falciparum histidine-rich protein 2 immunocapture assay results for acute schistosomiasis caused by Schistosoma mekongi. *J Clin Microbiol* 2011; 49: 2331–2332.
64. Gillet P, Jacobs J, Ngoyi D *et al.* Specificity of malaria rapid diagnostic tests is affected by *Trypanosoma brucei gambiense* sleeping sickness. *Malar J* 2012; 11 (Suppl 1): 64
65. Iqbal J, Sher A, Rab A. Plasmodium falciparum histidine-rich protein 2-based immunocapture diagnostic assay for malaria: cross-reactivity with rheumatoid factors. *J Clin Microbiol* 2000; 38: 1184–1186.
66. Maltha J, Gillet P, Cnops L *et al.* Evaluation of the rapid diagnostic test SDFK40 (Pf-pLDH/pan-pLDH) for the diagnosis of malaria in a non-endemic setting. *Malar J* 2011; 10: 7
67. Chiodini PL, Bowers K, Jorgensen P *et al.* The heat stability of *Plasmodium* lactate dehydrogenase-based and histidine-rich protein 2-based malaria rapid diagnostic tests. *Trans R Soc Trop Med Hyg* 2007; 101: 331–337.
68. Gillet P, Maltha J, Hermans V, Ravinetto R, Bruggeman C, Jacobs J. Malaria rapid diagnostic kits: quality of packaging, design and labelling of boxes and components and readability and accuracy of information inserts. *Malar J* 2011; 10: 39
69. World Health Organization *The Use of Rapid Malaria Diagnostic Tests, Second Edition* 2006.
70. Asiimwe C, Kyabayinze DJ, Kyalisiima Z *et al.* Early experiences on the feasibility, acceptability, and use of malaria rapid diagnostic tests at peripheral health centres in Uganda-insights into some barriers and facilitators. *Implement Sci* 2012; 7: 5
71. Hermans V, Monzote L, Van den Sande B *et al.* Assessment of the knowledge of graphical symbols labelled on malaria rapid diagnostic tests in four international settings. *Malar J* 2011; 10: 331
72. Barbe B, Gillet P, Beelaert G, Fransen K, Jacobs J. Assessment of desiccants and their instructions for use in rapid diagnostic tests. *Malar J* 2012; 13: 11
73. Harvey SA, Jennings L, Chinyama M, Masaninga F, Mulholland K, Bell DR. Improving community health worker use of malaria rapid diagnostic tests in Zambia: package instructions, job aid and job aid-plus-training. *Malar J* 2008; 7: 160
74. Rennie W, Phetsouvanh R, Lupisan S *et al.* Minimising human error in malaria rapid diagnosis: clarity of written instructions and health worker performance. *Trans R Soc Trop Med Hyg* 2007; 101: 9–18.
75. Chinkhumba J, Skarbinski J, Chilima B *et al.* Comparative field performance and adherence to test results of four malaria rapid diagnostic tests among febrile patients more than 5 years of age in Blantyre, Malawi. *Malar J* 2010; 9: 209

76. Hamer DH, Ndhlovu M, Zurovac D *et al.* Improved diagnostic testing and malaria treatment practices in Zambia. *JAMA* 2007; 23: 297
77. Reyburn H, Mbakilwa H, Mwangi R *et al.* Rapid diagnostic tests compared with malaria microscopy for guiding outpatient treatment of febrile illness in Tanzania: randomised trial. *BMJ* 2007; 24: 334
78. Rowe AK, de Leon GF, Mihigo J, Santelli AC, Miller NP, Van-Dunem P. Quality of malaria case management at outpatient health facilities in Angola. *Malar J* 2009; 8: 275
79. Skarbinski J, Ouma PO, Causer LM *et al.* Effect of malaria rapid diagnostic tests on the management of uncomplicated malaria with artemether-lumefantrine in Kenya: a cluster randomized trial. *Am J Trop Med Hyg* 2009; 80: 919–926.
80. D'Acremont V, Kahama-Maro J, Swai N, Mtasiwa D, Genton B, Lengeler C. Reduction of anti-malarial consumption after rapid diagnostic tests implementation in Dar es Salaam: a before-after and cluster randomized controlled study. *Malar J* 2011; 10: 107
81. Mubi M, Janson A, Warsame M *et al.* Malaria rapid testing by community health workers is effective and safe for targeting malaria treatment: randomised cross-over trial in Tanzania. *PLoS ONE* 2011; 6: e19753
82. Senn N, Rarau P, Manong D *et al.* Rapid diagnostic test-based management of malaria: an effectiveness study in Papua New Guinean infants with Plasmodium falciparum and Plasmodium vivax malaria. *Clin Infect Dis* 2012; 1: 54
83. Nadjm B, Mtove G, Amos B *et al.* Severe febrile illness in adult hospital admissions in Tanzania: a prospective study in an area of high malaria transmission. *Trans R Soc Trop Med Hyg* 2012; 106: 688–695.
84. Bottieau E, Gillet P, De Weggheleire A, Stokx J, Das Dorres Mosse C, Jacobs J. Treatment practices in patients suspected of malaria in the Provincial Hospital of Tete, Mozambique. *Trans R Soc Trop Med Hyg* 2013.
85. Faucher JF, Makoutode P, Abiou G *et al.* Can treatment of malaria be restricted to parasitologically confirmed malaria? A school-based study in Benin in children with and without fever. *Malar J* 2010; 9: 104
86. Njama-Meya D, Clark TD, Nzarubara B, Staedke S, Kamya MR, Dorsey G. Treatment of malaria restricted to laboratory-confirmed cases: a prospective cohort study in Ugandan children. *Malar J* 2007; 6: 7
87. Bisoffi Z, Sirima SB, Meheus F *et al.* Strict adherence to malaria rapid test results might lead to a neglect of other dangerous diseases: a cost benefit analysis from Burkina Faso. *Malar J* 2011; 10: 226
88. Graz B, Willcox M, Szeless T, Rougemont A. "Test and treat" or presumptive treatment for malaria in high transmission situations? A reflection on the latest WHO guidelines. *Malar J* 2011; 10: 136
89. Nadjm B, Amos B, Mtove G *et al.* WHO guidelines for antimicrobial treatment in children admitted to hospital in an area of intense Plasmodium falciparum transmission: prospective study. *BMJ* 2010; 340: c1350
90. Mori M, Ravinetto R, Jacobs J. Quality of medical devices and in vitro diagnostics in resource-limited settings. *Trop Med Int Health* 2011; 16: 1439–1449.
91. Peeling RW, Mabey D. Point-of-care tests for diagnosing infections in the developing world. *Clin Microbiol Infect* 2010; 16: 1062–1069.
92. World Health Organization *Universal access to malaria diagnostic testing An operational manual* 2011.
93. Chanthap L, Ariey F, Socheat D, Tsuyuoka R, Bell D. Low-technology cooling box for storage of malaria RDTs and other medical supplies in remote areas. *Malar J* 2010; 9: 31
94. Moonasar D, Goga AE, Frean J, Kruger P, Chandramohan D. An exploratory study of factors that affect the performance and usage of rapid diagnostic tests for malaria in the Limpopo Province, South Africa. *Malar J* 2007; 6: 74
95. Gillet P, Mori M, Van Den Ende J, Jacobs J. Buffer substitution in malaria rapid diagnostic tests causes false-positive results. *Malar J* 2010; 9: 215
96. Umbers AJ, Aitken EH, Rogerson SJ. Malaria in pregnancy: small babies, big problem. *Trends Parasitol* 2011; 27: 168–175.
97. Kattenberg JH, Ochodo EA, Boer KR, Schallig HD, Mens PF, Leeflang MM. Systematic review and meta-analysis: rapid diagnostic tests versus placental histology, microscopy and PCR for malaria in pregnant women. *Malar J* 2011; 10: 321
98. Dondorp AM, Desakorn V, Pongtavornpinyo W *et al.* Estimation of the total parasite biomass in acute falciparum malaria from plasma PfHRP2. *PLoS Med* 2005; 2: e204
99. Hendriksen IC, Mwanga-Amumpaire J, von SL *et al.* Diagnosing severe falciparum malaria in parasitaemic African children: a prospective evaluation of plasma PfHRP2 measurement. *PLoS Med* 2012; 9: e1001297
100. Mayor A, Moro L, Aguilar R *et al.* How hidden can malaria be in pregnant women? Diagnosis by microscopy, placental histology, polymerase chain reaction and detection of histidine-rich protein 2 in plasma. *Clin Infect Dis* 2012; 54: 1561–1568.
101. Kattenberg JH, Tahita CM, Versteeg IA *et al.* Evaluation of antigen detection tests, microscopy, and polymerase chain reaction for diagnosis of malaria in peripheral blood in asymptomatic pregnant women in Nanoro, Burkina Faso. *Am J Trop Med Hyg* 2012; 87: 251–256.
102. McMorrow ML, Aidoo M, Kachur SP. Malaria rapid diagnostic tests in elimination settings–can they find the last parasite?. *Clin Microbiol Infect* 2011; 17: 1624–1631.
103. Abdul-Ghani R, Al-Mekhlafi AM, Karanis P. Loop-mediated isothermal amplification (LAMP) for malarial parasites of humans: would it come to clinical reality as a point-of-care test?. *Acta Trop* 2012; 122: 233–240.
104. UNITAID. Malaria diagnostic technology landscape 2011.
105. Hopkins H, Oyibo W, Luchavez J *et al.* Blood transfer devices for malaria rapid diagnostic tests: evaluation of accuracy, safety and ease of use. *Malar J* 2011; 10: 30
106. Seidahmed OM, Mohamedein MM, Elsir AA, Ali FT, Malik eF, Ahmed ES. End-user errors in applying two malaria rapid diagnostic tests in a remote area of Sudan. *Trop Med Int Health* 2008; 13: 406–409.
107. Tavrow P *Using Quality Design To Improve Malaria Rapid Diagnostic Tests in Malawi* Bethesda: USAID; 2000.
108. Gillet P Malaria Rapid Diagnostic Tests: Laboratory aspects in the diagnostic setting, Maastricht University; 2011.
109. World Health Organization *How to use a Rapid Diagnostic Test (RDT): A guide for training at a village and clinic level* 2008.
110. Millipore Corporation Rapid lateral flow test strips Considerations for product development Bilerica: Millipore; 2008.
111. World Health Organization *Regional Office for the Western Pacific 2003 Malaria Rapid Diagnosis*: Making it Work Meeting report 20-23 January 2003 Manila, the Philippines 2003.