Implementation of a malaria rapid diagnostic test in a rural setting of Nanoro, Burkina Faso: from expectation to reality

François Kiemde1,2,3*, Marc Christian Tahita1, Massa dit Achille Bonko1,2, Petra F. Mens2, Halidou Tinto1, Michael Boele van Hensbroek3 and Henk D. F. H. Schallig2

Abstract

Background: Malaria rapid diagnostic tests (RDTs) are nowadays widely used in malaria endemic countries as an alternative to microscopy for the diagnosis of malaria. However, quality control of test performance and execution in the field are important in order to ensure proper use and adequate diagnosis of malaria. The current study compared the performance of a histidine-rich protein 2-based RDT used at peripheral health facilities level in real life conditions with that performed at central reference laboratory level with strict adherence to manufacturer instructions.

Methods: Febrile children attending rural health clinics were tested for malaria with a RDT provided by the Ministry of Health of Burkina Faso as recommended by the National Malaria Control Programme. In addition, a blood sample was collected in an EDTA tube from all study cases for retesting with the same brand of RDT following the manufacturer’s instructions with expert malaria microscopy as gold standard at the central reference laboratory. Fisher exact test was used to compare the proportions by estimating the p-value ($p \leq 0.05$) as statistically significant.

Results: In total, 407 febrile children were included in the study and malaria was diagnosed in 59.9% (244/407) of the cases with expert malaria microscopy. The sensitivity of malaria RDT testing performed at health facilities was 97.5% and comparable to that achieved at the laboratory (98.8%). The number of malaria false negatives was not statistically significant between the two groups ($p = 0.5209$). However, the malaria RDT testing performed at health facilities had a specificity issue (52.8%) and was much lower compared to RDT testing performed at laboratory (74.2%). The number of malaria false positives was statistically significantly different between the two groups ($p = 0.0005$).

Conclusion: Malaria RDT testing performed at the participating rural health facilities resulted in more malaria false positives compared to those performed at central laboratory. Several factors, including storage and transportation conditions but also training of health workers, are most likely to influence test performance. Therefore, it is very important to have appropriate quality control and training programmes in place to ensure correct performance of RDT testing.

Keywords: Malaria, HRP-2, RDT, Microscopy, Sensitivity and specificity
Background

The National Malaria Control Programme (NMCP) guidelines in Burkina Faso recommend that all suspicious malaria cases should be confirmed using either a RDT or light microscopy (if available) [1, 2]. Microscopy detecting Plasmodium parasites in Giemsa-stained thick or thin blood slides still remains the gold standard for malaria diagnosis [3]. The sensitivity and specificity of microscopy is however depending on the quality of the blood films, maintenance of the microscopy and training of the microscopists. Therefore, in many peripheral health settings RDTs have been introduced to fill this gap [4]. Malaria RDTs are in principle easy to perform in the field outside of a conventional laboratory, do not need much training to be performed, are relative cheap, and give a diagnostic result within 15 min [5].

The decision of many countries of sub-Saharan Africa (SSA), including Burkina Faso, to select malaria rapid diagnostic test detecting Plasmodium falciparum-specific histidine-rich protein 2 (PfHRP2) for diagnosis malaria is based on the high sensitivity and specificity reported by the World Health Organization (WHO) and Foundation for Innovative New Diagnostics (FIND) malaria RDT evaluation programme [6]. Secondly, PfHRP2-based RDTs are reported to have a good thermal and humid stability compared to tests targeting Plasmodium-specific parasite lactate dehydrogenase (pLDH) [7]. Indeed, exposure of PfHRP2 RDT to high temperature (up to 45°C) over a prolonged period of up to 24 months did not affect the quality of the test [6]. However, the influence of a very high temperature (>45 °C; which is common in several SSA countries) and humidity (>65%) are not documented. In addition, transport and storage conditions and operator performance have been reported to influence RDT performance [8–11]. Thirdly, the issue of persisting HRP2 antigens after successful treatment has been raised as a major factor contributing to reduce the specificity of PfHRP2-based RDTs [12–19]. Finally, there is also increasing concern with respect to reported false positive diagnosis by PfHRP2 RDT in particular in low malaria transmission settings [20].

These concerns warrant close monitoring of the performance of RDTs under field conditions. The objective of the present study was to assess the performance of the recommended HRP2 RDT by the Burkina Faso NMCP executed at peripheral level compared with the performance of the same brand of RDT in controlled conditions at a central reference laboratory with strict adherence to the manufacturer instructions.

Methods

Study design

The study was conducted between April and October 2016 in the health district of Nanoro, which is located at approximately 100 km from Ouagadougou, the capital city of Burkina Faso. Malaria is endemic with the transmission peak occurring between July and November and P. falciparum is the predominant malaria parasite [21]. The study was conducted as part of a large project aiming to assess fever aetiologies in Nanoro [22]. Briefly, children under 5 years with an axillary temperature ≥37.5 °C presenting at one of the participating health facilities were asked to participate. After obtaining the consent from parent or legal guardian, the participant was enrolled in the study. The malaria RDT used to screen febrile children at recruitment in the health facilities during the study period was the HRP2-based RDT specific to P. falciparum (SD Ag Bioline Pf: Standard Diagnostics, Hagal-Dong, Korea). Information on lot number and expiration date was not collected. The result of malaria RDT testing in the health facilities was recorded on a case record form.

After inclusion, a blood sample was collected for each child in ethylene diamine tetra acetic acid (EDTA) tube, transported under cold conditions in an ice-box at the laboratory of Clinical Research Unit of Nanoro (CRUN). Expert malaria microscopy was performed by expert laboratory technician from blood collected in EDTA tube before stored at −20 °C until retesting. The retesting is done at the laboratory of CRUN with experimented technician with a PfHRP2 RDT of the same manufacturer (SD Ag Bioline Pf: Standard Diagnostics, Hagal-Dong, Korea; Lot number: 05EDC002A; Expiration date: 01/03/2019). The RDTs are transported and stored according to the manufacturer’s instructions. Standard Operating Procedures (SOPs) for ordering, transportation, storage and performing malaria RDTs are in place at CRUN. Blood samples are thawed at room temperature before retesting.

Laboratory procedures

For retesting in the CRUN laboratory, the blood sample was thawed at room temperature and the diagnostic test was performed according to the manufacturer’s instructions. One trained technician performed the RDT, but the result was read by two technicians and in case of a discordant opinion a third reader would be consulted. The laboratory technicians who repeated the malaria RDT were blinded from the malaria RDT results obtained at the health facilities and the RDT test results were reported on separate case record forms.
Malaria slide reading was performed by expert microscopists who are participating in an external quality programme and only certified microscopists were allowed to read the slides. The limit of detection (LoD) of this expert microscopy was 10 parasites per µl [23]. Thin films were fixed with methanol and blood slides were stained with 3% Giemsa solution (pH 7.2) for identification and quantification of asexual *P. falciparum* and other *Plasmodium* species. Parasites densities were determined by counting the number of asexual parasites per 200 white blood cells, and calculating per µl of blood by assuming the number of white blood cells to be at 8000 per µl. Thick blood smears were considered negative when the examination of 200 fields per thick film did not reveal the presence of any asexual parasites. Each blood slide was read by two independent expert readers, and in case of discordance (positive vs negative, different in *Plasmodium* species, difference in parasite density > Log10 or ratio > 2 in case of parasite density ≤ 400/µl or > 400/µl, respectively), the blood slide was read by a third independent reader. Positive microscopy results were recorded as the geometric means of the two reader’s results or the geometric means of the two geometrically closest reading in case of third reading. These results were expressed as asexual parasites per µl by using the patient’s white blood cell (WBC) count. A selection of slides (5%) was re-read by an independent expert microscopist for quality assurance. All microscopists were blinded from the results obtained with the different malaria RDTs.

**Ethical approval**

The study was approved by the National Ethical Committee in Health Research, Burkina Faso (Deliberation N°2014-11-130). The study was also approved by the health district authorities and community leaders of different villages before implementation.

**Data analysis**

Double entered data was done using Excel 2016. The data analysis was done with R software version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). For the quantitative data, the descriptions were performed by using mean or median respectively. Proportion was used to describe qualitative data. The evaluation of the performance of the malaria RDT performed by nurses at health centres level was done by comparing the results of *PfHRP2* tests performed in the field by health facilities nurses to those repeated in CRUN laboratory by trained technicians. Proportions were used to present the concordance and discordance between the two tests performed in malaria positive and negative groups. Fisher exact test was used to compare the proportions by estimating the p-value (p ≤ 0.05) as statistically significant. Agreement between each RDT test and microscopy and between the two RDTs tests was determined by calculating Kappa (κ) values with 95% confidence intervals by using GraphPad software (https://www.graphpad.com/quickcalcs/).

**Results**

**Description of the study population**

In total, 407 children were included in the study. The median age of enrolled children was 23.0 months [IQR (interquartile): 12.0–36.0] and the mean axillary temperature was 37.7 °C (standard deviation: 0.78 °C). Males represented 56.8% (231/407) of the study population (Table 1).

**Results of RDT testing and expert microscopy**

The number of positive cases of malaria determined by performing a *PfHRP2* RDT at the health facilities level by nurses (HF-*PfHRP2*) was 77.4% (315/407). No test failures were reported at health facilities. Retesting the collected blood samples in the laboratory of CRUN (Lab-*PfHRP2*) revealed that 69.3% (282/407) was RDT positive (Table 1). No invalid tests were observed at CRUN and there was no need for a third opinion as all initial readings were in agreement.

The number of *P. falciparum* malaria microscopy positive slides as assessed by expert microscopists was 59.9% (224/407), with a geometric mean and median parasite density of 22,839.4 parasites/µl (range 32–586,250 parasites/µl) and 39,847 (7828–95,369) respectively. Co-infections were found in 5 cases: there were 4 co-infections with *Plasmodium malariae* and 1 case with *Plasmodium ovale*. There was no need for a third reader as the two expert technicians agreed on the initial microscopy result.

**Table 1 Baseline characteristic of study population**

| Characteristics                  | N = 407 |
|----------------------------------|---------|
| Age in months, median (IQR)      | 23.0 (12.0–36.0) |
| Male, n (%)                      | 231 (56.8) |
| Axillary temperature °C, mean (SD)| 37.7 (0.78) |
| Parasites/µl, geometric mean (min-max) | 22,839.4 (32–586,250) |
| Malaria positive by expert microscopy, n (%) | 244 (59.9) |
| Malaria positive health facilities RDT-*PfHRP2*, n (%) | 315 (77.4) |
| Malaria positive laboratory RDT-*PfHRP2*, n (%) | 282 (69.3) |
Performance of malaria RDT detecting PfHRP2 performed at the health facilities level by nurses and at the laboratory level by trained technicians compared to expert microscopy (gold standard)

| Performance characteristic | HF-PfHRP2 n (%) | Lab-PfHRP2 n (%) | p-value |
|-----------------------------|-----------------|-----------------|---------|
| True positive               | 238 (58.5)      | 240 (59.0)      | 0.8848  |
| True negative               | 86 (21.1)       | 121 (29.7)      | 0.0048  |
| False positive              | 77 (18.9)       | 42 (10.3)       | 0.0005  |
| False negative              | 06 (1.5)        | 04 (1.0)        | 0.5209  |

Table 2 Performance of PfHRP2-based rapid diagnostic test performed by study nurses at health facilities (HF-PfHRP2) or PfHRP2-based rapid diagnostic test performed at the central microbiology laboratory (Lab PfHRP2) by trained technicians compared with expert microscopy (gold standard)

| Diagnostic performance characteristic | HF-PfHRP2 | 95% CI | Lab-PfHRP2 | 95% CI |
|----------------------------------------|-----------|--------|------------|--------|
| Sensitivity                             | 97.5 (238/244) | 94.7–99.1 | 98.4 (240/244) | 95.9–99.6 |
| Specificity                             | 52.8 (86/163)  | 44.8–60.6 | 74.2 (121/163) | 66.8–80.8 |
| Positive predictive value               | 75.6 (238/315) | 72.4–78.5 | 85.1 (240/282) | 81.5–88.1 |
| Negative predictive value               | 93.5 (86/92)  | 86.5–97.0 | 96.8 (121/125) | 91.9–98.8 |

Table 3 Diagnostic accuracy of PfHRP2-based rapid diagnostic test performed by study nurses at health facilities (HF-PfHRP2) and PfHRP2-based rapid diagnostic test performed at the central microbiology laboratory (Lab PfHRP2) by trained technicians compared using expert microscopy as gold standard

Table 4 Agreement between the different diagnostic procedures
Discussion

Rapid diagnostic tests for malaria are widely implemented by National Malaria Control Programmes in endemic countries, including Burkina Faso, in order to meet the WHO requirement of confirming a malaria infection before starting a treatment [1, 2]. RDTs are hence increasingly replacing (expert) microscopy in many settings, but there is a concern about the diagnostic accuracy of HRP2-based RDTs. Several studies reported a lower sensitivity of RDTs compared to expert microscopy when the parasitaemia is < 200 parasites/µl [24]. This situation is exacerbated by the fact that PfHRP2 polymorphisms are being reported and that certain deletions in this gene may negatively affect RDT performance [25–27]. These PfHRP2 gene deletions have so far not been found in Burkina Faso, but do occur in neighbouring Mali [25]. However, a sensitivity issue was not observed in the present study. There was no significant difference in test sensitivity when the RDT was performed by the two different groups of operators (nurses in the rural health facilities compared to trained laboratory technicians). Importantly, the RDT sensitivity and NPV achieved by both groups almost reached the level of expert microscopy. Only few false negative results were reported with the employed RDTs.

In contrast, the specificity (and subsequently the PPV) of the RDT was worrying low (52.8%) when the test was performed by the nurses at the health facilities level. This is also reflected in the observed agreement between the tests. Overall, the agreement between expert microscopy and Lab-HRP2 was good, but moderate between HF-RDT and expert microscopy. This could be explained by the fact that the HF-RDT was more often false positive. In general, the specificity of the HRP2-based RDTs is being questioned particularly under low transmission conditions [20]. This is supported by one of previous studies conducted in the same study area in which a high prevalence of false positive RDT results was reported during the dry season (April–May; low transmission) [22]. However, the present study was mainly conducted during the rainy season (June–October; high transmission season) but still the number of false positive tests was almost two-fold higher at the participating health facilities compared to the laboratory of CRUN. It is more obvious that the number of false positive cases could thus be higher if the study was conducted mainly during the dry season.

HRP2 persistence after a successful treatment is often used as an explanation for the lower specificity of RDTs that are based on the detection of this specific antigen [12–19]. This can however not explain the difference in the test performance observed between the two different groups of operators (i.e. health facility nurses vs laboratory technicians). Several other factors can influence the RDT performance including incorrect test execution and reading of RDT results by health facility nurses whilst performing the test [9, 10]. Also operator errors such as incorrect application of the blood sample or running buffer on the test device, substituting test kit buffer solution with other liquids such as normal saline, diluted water, tap water or buffer from different kits/lots/batches or faulty test devices can affect the test performance by health facility nurses [11]. A very long-reading time could also explain the high positive rate of false positive at health levels. Some non-specific binding or interaction with other immunological or infection factors, such as rheumatoid factors, hepatitis C, schistosomiasis, toxoplasmosis, dengue, leishmaniasis, Chagas’ disease and human African trypanosomiasis can lead to a malaria false positive reaction on a HRP2-based RDT, though considered to be rare [6, 28–36]. It is, therefore, crucial to ensure adequate training of the health workers who perform the RDT and periodically monitor the execution of malaria RDT by the health workers [37].

According to the WHO testing report and the RDT manufacturer information note, the P. falciparum specific HRP2-based RDT can stand up to 40 °C for 24 months [6]. However, in Burkina Faso, the mean maximum temperature can reach 45 °C in the dry season [38]. The participating health facilities in this study had no air-conditioning system or temperature and
humidity monitoring system in their store room. This can severely affect the test performance as previously reported [8, 39, 40]. Moreover, periodical quality checks of the RDT at the health facilities are not in place. The above-mentioned issues should all be addressed when implementing the malaria PfHRP2 RDT.

Finally, the blood specimen used to perform the malaria RDTs in the field was a capillary sample and the one used to perform malaria RDTs in the central laboratory was from venous blood. It has been reported that the sensitivity of malaria tests (i.e. microscopy) depend on the site of blood collection, in particular in asymptomatic malaria cases. Capillary blood tends to be more sensitive than venous blood [41]. However, the clinical symptoms of malaria infection, including fever, occur in synchrony with the rupture of infected erythrocytes and the release of these erythrocytes and malaria debris in circulating blood [42, 43]. So, it is obvious that symptomatic malaria, which was studied in the present research, will be detectable in capillary blood as well as venous blood.

**Conclusion**

Rapid diagnostic tests are a valuable tool for the diagnosis of malaria in settings where expert microscopy is not available. However, some external factors could negatively influence the performance of these RDTs in the field. As long as these factors remain, causes of fever might not be correctly diagnosed and results in inappropriate prescription of anti-malarials and antibiotics in fear of overlooking a treatable infection.

**Abbreviations**

CRUN: Clinical Research Unit of Nanoro; EDTA: ethylene diamine tetra acetic acid; FIND: Foundation for Innovative New Diagnostics; HF: health facility; HRP2: histidine-rich protein; Lab: laboratory; LoD: limit of detection; Pf: Plasmodium falciparum; NMCP: National Malaria Control Programme; NPV: negative predictive value; pLDH: Plasmodium-specific parasite lactate dehydrogenase; PPV: positive predictive value; RDT: rapid diagnostic test; SD: standard diagnostic; SSA: sub-Saharan Africa; WBC: white blood cells; WHO: World Health Organization.

**Authors’ contributions**

FK, HS, PM, HT and MBvH conceived and designed the study. FK, MT and MB supervised patient inclusion, signature of informed consent and diagnostic specimen collection by study nurses. FK, MT and MB performed/supervised the laboratory analyses (malaria microscopy and the retest of malaria RDTs). FK analyzed the data under the supervision of a biostatistician. FK and HS drafted the manuscript and all authors commented on draft versions. All authors read and approved the final manuscript.

**Acknowledgements**

We would like to thank the study staff of the rural health facilities and the hospital OMA Saint Camille de Nanoro for their valuable contributions to the work. We are indebted to the children and their parents or legal guardians for their participation in the study.

**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

The study protocol was reviewed and approved by the National Ethical Committee in Health Research, Burkina Faso (Deliberation N°2014-11-130). Written informed consent for the participation of the children was obtained from parents or legal guardians prior to enrolment in the study.

**Funding**

The work was financially supported by a grant from the Netherlands Organization for Health Research and Development (ZonMw), project 205300005, RAPDIF: a rapid diagnostic test for undifferentiated fevers.

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**References**

1. Programme National de Lutte contre le Paludisme. Directives nationales pour la prise en charge du paludisme dans les formations sanitaires du Burkina Faso. Ouagadougou; 2014.
2. WHO. Guidelines for the treatment of malaria. 2nd ed. Geneva: World Health Organization; 2010. p. 197.
3. Moody A. Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev. 2002;15:66–78.
4. Maltha J, Gillet P, Bottieau E, Cronps L, van Esbroeck M, Jacobs J. Evaluation of a rapid diagnostic test (CareStart™ Malaria HRP-2/pLDH (Pf/pan) Combo Test) for the diagnosis of malaria in a reference setting. Malar J. 2010;9:171.
5. Mathison BA, Pritt BS. Update on malaria diagnostics and test utilization. J Clin Microbiol. 2017;55:2009–17.
6. WHO. Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: round 6 (2014–2015). Geneva: World Health Organization; 2015.
7. WHO. World malaria report 2011. Geneva: World Health Organization; 2011.
8. Albertini A, Lee E, Coulibaly SO, Sleshi M, Faye B, Matong ML, et al. Malaria rapid diagnostic test transport and storage conditions in Burkina Faso, Senegal, Ethiopia and the Philippines. Malar J. 2012;11:406.
9. Rennie W, Phetsouvanh R, Lupisan S, Vanisaveth V, Hongvanthong B, Phompida 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.
10. Masanja IM, Maganga M, Sumari D, Luchu N, Udhayakumar V, McMorrow M, et al. Evaluation of two quality assurance approaches for malaria rapid diagnostic tests in peripheral health facilities in rural tanzania. Am J Trop Med Hyg. 2012;Conference:255.
11. 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.
12. Tijia E, Suprianto S, McBroom J, Currie BJ, Anstey NM. Persistent ICT malaria Pf/Pv Panmalarial 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. 2001;39:1025–31.

13. Kattenberg JH, Tahitia CM, Vensteeg IA, Tinto H, Tracee-Coulibaly M, Schallig HDFH, et al. Antigen persistence of rapid diagnostic tests in pregnant women in Nanoro, Burkina Faso, and the implications for the diagnosis of malaria in pregnancy. Trop Med Int Health. 2012;17:550–7.

14. Maltha J, Guiraud I, Lompo P, Kabore B, Gillet P, Van Geet C, et al. Accuracy of PfHRP2 versus Pf-pLDH antigen detection by malaria rapid diagnostic tests in hospitalized children in a seasonal hyperendemic malaria transmission area in Burkina Faso. Malar J. 2014;13:20.

15. Hendrikse ICE, Mtove G, Pedro AJ, Gomes E, Silamut K, Lee SJ, et al. Evaluation of a PfHRP2 and a pLDH-based rapid diagnostic test for the diagnosis of severe malaria in 2 populations of african children. Clin Infect Dis. 2011;52:1100–7.

16. Barber BE, Williams T, Grigg MJ, Piera K, Yeo TW, Anstey NM. Evaluation of PfHRP2 and Pf-pLDH antigen detection for malaria rapid diagnostic tests: the case of human African trypanosomiasis. PLoS Negl Trop Dis. 2013;7:e2180.

17. Lee J-H, Jang JW, Cho CH, Kim JY, Han ET, Yun SG, et al. False positivity of non-targeted infections in malaria rapid diagnostic tests: the case of human African trypanosomiasis. PLoS Negl Trop Dis. 2013;7:e2180.

18. Ochola LB, Younatsou P, Smith T, Mabaso ML, Newton CR. The reliability of diagnostic techniques in the diagnosis and management of malaria in the absence of a gold standard. Lancet Infect Dis. 2006;6:582–8.

19. Wilson ML. Malaria rapid diagnostic tests. Clin Infect Dis. 2012;54:1637–41.

20. Diakolou MA, Diongue K, Ndaiye M, Gaye A, Dembe A, Badiane AS, et al. Evaluation of CareStart™ Malaria HRP2/pLDH (Pf/pan) Combo Test in a malaria low transmission region of Senegal. Malar J. 2017;16:328.

21. District Sanitaire de Nanoro. Plan Strategique 2016 du District Sanitaire de Nanoro; 2016.

22. Kiemde F, Bonko MTA, Tahita CM, Lompo P, Rouamba T, Tinto H, et al. Accuracy of a PfHRP2/pLDH-specific histidine-rich protein 2 rapid diagnostic test in the context of the presence of non-malaria fevers, prior anti-malarial use and seasonal malaria transmission. Malar J. 2017;16:294.

23. Tinto H, Ouedraogo JB, Traore M, Guiguemde TR. [Parastological resistance of Plasmodium falciparum to antimalarial drugs: what physicians should keep in mind. Sante. 2004;14:69–73.

24. McMorrow ML, Aidoo M, Kashur SP. Malaria rapid diagnostic tests in elimination settings-Can they find the last parasite? Clin Microbiol Infect. 2017;23:1634–5.

25. 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;38:1184–6.

26. Kiemde F, Bonko MTA, Tahita CM, Lompo P, Rouamba T, Tinto H, et al. Evaluation of CareStart™ Malaria HRP2/pLDH (Pf/pan) Combo Test in a malaria low transmission region of Senegal. Malar J. 2017;16:328.

27. Kiemde F, Bonko MTA, Tahita CM, Lompo P, Rouamba T, Tinto H, et al. Accuracy of a PfHRP2/pLDH-specific histidine-rich protein 2 rapid diagnostic test in the context of the presence of non-malaria fevers, prior anti-malarial use and seasonal malaria transmission. Malar J. 2017;16:294.

28. Kiemde F, Bonko MTA, Tahita CM, Lompo P, Rouamba T, Tinto H, et al. Accuracy of a PfHRP2/pLDH-specific histidine-rich protein 2 rapid diagnostic test in the context of the presence of non-malaria fevers, prior anti-malarial use and seasonal malaria transmission. Malar J. 2017;16:294.

29. Sandeu MM, Bayibéki AN, Tchiefo MT, Abate L, Gimonneau G, Awono-Ambéné PH, et al. Do the venous blood samples replicate malaria parasite densities found in capillary blood? A field study performed in naturally-infected asymptomatic children in Cameroon. Malar J. 2017;16:345.

30. Schofield L, Hackett F. Signal transduction in host cells by a glycosylphosphatidylinositol toxin of malaria parasites. J Exp Med. 1993;177:145–53.

31. Murray CK, Gasser RA, Magill AJ, Miller RS. Update on rapid diagnostic testing for malaria. Clin Microbiol Rev. 2008;21:97–110.

32. Sandeau MM, Bayibéki AN, Tchiefo MT, Abate L, Gimonneau G, Awono-Ambéné PH, et al. Do the venous blood samples replicate malaria parasite densities found in capillary blood? A field study performed in naturally-infected asymptomatic children in Cameroon. Malar J. 2017;16:345.

33. Gillet P, Mumba Ngoyi D, Lukuka A, Kande V, Atua B, van Griensven J, et al. False positivity of non-targeted infections in malaria rapid diagnostic tests: the case of human African trypanosomiasis. PLoS Negl Trop Dis. 2013;7:e2180.

34. Lee J-H, Jang JW, Cho CH, Kim JY, Han ET, Yun SG, et al. False-positive results for rapid diagnostic tests for malaria in patients with rheumatoid factor. J Clin Microbiol. 2014;52:3784–7.

35. Grobusch M, Alpermann U, Schwenke S, Jelinek T, Warhurst D, Vugt M. False-positive rapid tests for malaria in patients with rheumatoid factor. Lancet. 1999;353:297.

36. Laferi H, Kandel K, Pichler H. False positive dipstick test for malaria. N Engl J Med. 1997;337:1635–6.

37. Ruizendaal E, Schallig HDFH, Scott T, Traore-Coulibaly M, Bradley J, Lompo P, et al. Evaluation of malaria screening during pregnancy with rapid diagnostic tests performed by community health workers in Burkina Faso. Am J Trop Med Hyg. 2017;97:1190–7.

38. Gnouregna Ubame Umayoog. Etat de reference des observatoires de la mere d’Oursi et de la reserve de la biosphere de la mare aux hippopotames; 2011.

39. Chiidini PL, Bowers K, Jorgensen P, Barnwell JW, Grady KK, Luchavez J, 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;101:331–7.

40. Murray CK, Gasser RA, Magill AJ, Miller RS. Update on rapid diagnostic testing for malaria. Clin Microbiol Rev. 2008;21:97–110.

41. Sandeau MM, Bayibéki AN, Tchiefo MT, Abate L, Gimonneau G, Awono-Ambéné PH, et al. Do the venous blood samples replicate malaria parasite densities found in capillary blood? A field study performed in naturally-infected asymptomatic children in Cameroon. Malar J. 2017;16:345.

42. Schofield L, Hackett F. Signal transduction in host cells by a glycosylphosphatidylinositol toxin of malaria parasites. J Exp Med. 1993;177:145–53.

43. Clark IA, Cowden WB. The pathophysiology of falciparum malaria. Pharcacol Ther. 2003;99:221–60.