HSI Health Sciences Investigations Journal

share

Send us an email: info@hsijournal.org

https://www.hsijournal.org

ISSN Online 2704-4890 | ISSN Print 2720-7609

Original Research Article

Online first publication

HSI Journal (2020) Volume 1(Issue 2): 86-92. http://doi.org/10.46829/hsijournal.2020.12.1.2.86-92

Diagnostic performance of HRP2-only malaria rapid diagnostic test in Ghanaian pregnant women with asymptomatic peripheral blood infection: the case of First Response® test kit

Joseph Osarfo [1*], Harry Tagbor [2], Michael Alifrangis [3], Pascal Magnussen [3]

[1]Ghana Health Service, Asante Mampong Municipal Hospital, Asante Mampong, Ashanti Region, Ghana;
[2]Department of Community Medicine, School of Medicine, University of Health and Allied Sciences, Ho, Ghana;
[3]Centre for Medical Parasitology, Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

Received August 2020; Revised September 2020; Accepted October 2020

Abstract

Background: The use of malaria rapid diagnostic test (RDT) enables targeted treatment that mitigates against the development of parasite drug resistance. With detection thresholds at ≥ 200 parasites/µL, their diagnostic performance in pregnant women may be challenging as asymptomatic infections with low parasite densities are common. Few data exist on the performance of histidine-rich protein-2 (HRP2) RDTs in Ghanaian pregnant women considering commonly occurring low parasite densities.

Objective: The study sought to contribute more knowledge on test performance on First Response® test kit to guide the continuous evaluation of HRP2 RDTs in pregnancy.

Methods: As part of an antimalarial drug trial in pregnancy, First Response® RDT results were compared to microscopy of peripheral blood slides in 1664 women. The diagnostic performance indicators were computed as proportions with 95% confidence intervals. The risk of having a positive RDT result was computed for age, gravidity and parasite density using binomial regression methods.

Results: Parasitaemia prevalence by microscopy was 5.71% (n = 95/1664) while that by RDT was 21.57% (n = 359/1664). Sensitivity was 82.11% (n=78/95), specificity was 82.09% (n=1288/1569), positive predictive value was 21.73% (n = 78/359), and the likelihood ratio for a positive test was 4.58. False-negative RDT results were recorded for low parasite densities as well as densities ≥ 200/ µL while false-positive results were recorded in 281 of 1664 tests. Primigravidae and younger aged women were more likely to have positive RDT results compared to multigravidae and women aged ≥ 30 yr.

Conclusion: The moderate sensitivity, specificity and other diagnostic parameters reported suggest the First Response® malaria RDT is useful for detecting peripheral parasitaemia in pregnant women but the use of HRP2-only RDTs is limited by the existence of parasites with HRP2 gene deletion. The use of RDTs based on combined antigens continues to be recommended. Further research is needed on RDT performance in pregnant women with declining malaria transmission.

Keywords: *Malaria rapid diagnostic performance, histidine-rich protein 2, Plasmodium falciparum, Ghana*

Open Access

INTRODUCTION

Malaria rapid diagnostic tests detect parasite antigens including histidine-rich protein-2 (HRP2) in *Plasmodium falciparum*, lactate dehydrogenase (LDH) and aldolase individually or combined in antigen-antibody reactions. Rapid diagnostic tests based on HRP2 reportedly have superior reliability though HRP2 can persist in bloodstream for up to 2 - 3 wk or longer after parasite clearance and complicate test result interpretation [1]. Nonetheless, the sensitivity of HRP2 RDTs is known to vary with varying parasite densities, sub-optimal storage and testing skills [2-3]. Studies have shown that deletion of *P. falciparum* HRP2 gene [4-8] may underlie false-negative RDT results and reduce sensitivity. The test performance at parasite density of 200/µL was deemed very important in the last round of the World Health Organization (WHO) testing [9]. Pregnant women often

* *Corresponding author*
Email: josarfo@yahoo.co.uk

have asymptomatic malaria infection characterized by low parasite densities in the peripheral blood due to placental sequestration [10,11]. With many standard RDTs having detection thresholds of about 100 parasites/µL to ≥ 200 parasites/µL, there may be challenges for identifying individuals with low-density infections [12,13]. This will likely have a greater impact on pregnant women compared to other vulnerable populations considering the adverse effects of pregnancy-associated malaria on mother, fetus and infant [14-16]. Subsequently, treatment to avoid maternal anaemia, low birth weight, preterm births and other complications may be denied. Compared to a polymerase chain reaction (PCR) or microscopy as a reference, HRP2-based assays in pregnant women have shown sensitivity ranging from 67.3% to 100% and specificity up to 97% for peripheral blood parasitaemia [17-19].

A combined HRP2/LDH RDT in West African pregnant women showed sensitivities of 91% in Burkina Faso, 89% in Ghana, 59% in the Gambia and 87% in Mali for *P. falciparum* with peripheral blood microscopy and PCR, individually or combined, as the standard [20]. In another study also in pregnancy, a similar combined HRP2/pan LDH RDT showed lower sensitivities of 75.7% and 55.8% respectively compared to PCR in Uganda and Burkina Faso [21]. Sensitivity and specificity are typically emphasized in RDT performance assessments though these have little clinical relevance as they reflect test results given that a disease is present or absent [22]. Of more clinical value and patient-centred are the predictive values. These predict an individual's chance of having a disease or otherwise given a positive or negative test result. The likelihood ratio is the probability of someone with the disease testing positive or negative compared to one without the disease [22,23]. There are few data on the reliability of HRP2-based RDTs in pregnant women in Ghana.

A literature review found only two published reports; One used a combined HRP2/LDH RDT compared to PCR or microscopy or both and reported a declining sensitivity ranging from 89% at study enrolment to 49% at delivery [20]. The reduction in sensitivity at delivery was thought to be due to lower parasite densities resulting from preventive and therapeutic malaria treatment during pregnancy. The second report reported sensitivity and specificity compared to PCR of 97.5% and 99.1% respectively [24]. The first study was conducted in a part of northern Ghana where malaria transmission is perennial. The second study was conducted in an area with seasonal malaria transmission and over a short period when parasitaemia prevalence and density tend to be high. The differences may account for the high variability in sensitivity reported. To contribute to knowledge on the performance of HRP2-only RDTs in detecting peripheral parasitaemia in pregnancy, the diagnostic performance of the First Response® malaria rapid diagnostic test, compared to light microscopy, was assessed as part of an antimalarial drug trial in pregnant women [25].

MATERIALS AND METHODS

Study design

The study was nested in an antimalarial drug trial in pregnant women with asymptomatic *P. falciparum* infection [25]. The trial was conducted in Bekwai and Bosomtwi, two contiguous districts in the Ashanti region in the middle forest belt of Ghana. Malaria transmission is perennial and of moderate-to-high intensity. In brief, second and third-trimester pregnant women of all gravidity and age ≥ 15 yr. accessing routine antenatal care services with no complaints of illness at Bekwai Government Hospital and St. Michael's Hospital in Bosomtwi were screened for *P. falciparum* parasitaemia using the First Response® malaria RDT (Premier Medical Corporation, India) and microscopy of thick and thin finger-prick blood films. The RDT was assessed and results read according to the manufacturer's instructions. A laboratory technologist blinded to the RDT results read the blood films under high power field using oil immersion. One hundred (100) high power fields were examined before a slide was declared negative. Parasite density was determined by counting the number of *P. falciparum* parasites against 200 white blood cells (WBC), assuming a WBC count of 8,000/µL of blood.

Sample size determination

A 12% prevalence of pregnant women with peripheral blood parasitaemia based on combined HRP2-based RDT and microscopy has been reported in the study area [25]. The Cochran's formula [26] was used $\frac{z^2pq}{d^2}$ at 95% confidence interval where z = 1.96, p is the proportion of pregnant women with peripheral blood parasitaemia determined using both HRP2 RDT and microscopy, q = (1-p) and d is the allowable margin of error of 1.6%

$$n = \frac{(1.96^2 \times 0.12 \times 0.88)}{0.016^2}$$

$$n = 1{,}584.66$$

The sample size was adjusted by 10% to 1,761 to account for RDT and blood slide pairs that may not ultimately contribute to final data analysis. This was then approximated to 1800.

Statistical analysis

The 1800 RDTs and blood slide pairs constituted 52% of the total number prepared in the trial (n = 3464) and were randomly selected using the randomization function in STATA 12 (Stata Corp, USA). The proportion of participants with or without parasitaemia, sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) and likelihood ratios for positive and negative tests were computed with 95% confidence interval (CI). The risk of having a positive RDT result was computed for participant characteristics including age and gravidity using binomial regression methods. Associations between outcome and independent variables were considered statistically significant if the $p \leq 0.05$.

Table 1: Participants characteristics and parasitological data

Variable	Description
Age in yr. (n = 1657)	
Mean ± SD	27.5 ± 5.9
Range	15 - 45
Age categories	
15-24	542 (32.7%)
25-34	865 (52.2%)
≥34	250 (15.1%)
Gravidity (n = 307)	
Median	1
Range	0 - 9
1	86 (28.0%)
2	80 (26.1%)
≥ 3	141 (45.9%)
Microscopy positive slides (n = 95)	
P. falciparum	80 (84.2%)
P. malariae	2 (2.1%)
P. falciparum / P. malariae	11 (11.6%)
P. ovale	2 (2.1%)
[a] Parasite density data (n = 91)	
Geometric mean Parasite density (95% CI)	1586.55/µL (961.32 - 2618.43)
Range	40 - 69680/µL
<1000/µL	44 (48.4%)
≥1000/µL	47 (51.66%)

*SD, standard deviation; n is total number assessed for a variable; [a] parasite density data was limited to blood slides with *P. falciparum*.

Table 2: Performance of First Response® malaria rapid diagnostic tests compared to peripheral film microscopy in pregnant women.

First Response® RDT	Microscopy		
	Negative	Positive	Total
Negative	1288	17	1305
Positive	281	78	359
Total	1569	95	1664
Percentage Sensitivity (95% CI)		82.1 (72.9, 89.2)	
Percentage Specificity (95% CI)		82.1 (80.0, 84.0)	
Percentage Positive Predictive Value (95% CI)		21.7 (17.6, 26.4)	
Percentage Negative Predictive Value (95% CI)		98.7 (97.9, 99.2)	
Area under Receiver Operating Characteristic Curve (95% CI)		0.82 (0.78, 0.86)	
Positive Likelihood Ratio (95% CI)		4.58 (3.98, 5.28)	
Negative Likelihood Ratio (95% CI)		0.22 (0.14, 0.34)	

*CI, confidence interval; RDT, rapid diagnostic tests.

RESULTS

Of the 1800 RDT vs peripheral blood film pairs, 1664 pairs were included in analysis. The blood films of the remaining 136 were judged to be of rather poor quality. About 21.57% (n = 359/1664) of RDTs showed positive test results while only 5.71% (n = 95/1664) of peripheral blood slides were positive. The prevalence of *P. falciparum* was 5.47% (n = 91/1664) based on microscopy with a geometric mean parasite density of 1587/µL (95% CI: 961 - 2618).

Table 1 shows the background characteristics and parasitological data for participants in the First Response® RDT diagnostic performance assessment. Table 2 shows the performance of First Response® malaria RDT compared to microscopy. Sensitivity was 82.11% (n=78/95), specificity was 82.09% (n=1288/1569) and the positive predictive value was 21.73% (n = 78/359). The area under the receiver operating characteristic curve (ROC) was 0.82 (Figure 1). Excluding four slides with *P. malariae* or *P. ovale* from the analysis did not change any of the above values significantly. Higher age groups showed statistically significant lower risks of a positive RDT result compared to those in the age group 15 - 19 yr. (Table 3). Women aged ≥ 30 yr. had a risk ratio of 0.29 (95% CI: 0.22 - 0.38; $p <$ 0.001). Similarly, multigravid women were less likely to show positive RDT results compared to primigravidae [relative risk (RR), 0.63; 95% CI: 0.45 - 0.87; $p = 0.006$). There was no significant difference between primigravidae and secundigravidae regarding the risk of a positive RDT outcome (RR, 0.69; 95% CI: 0.47, 1.01; $p = 0.059$). The association between age and the risk of a positive RDT result remained significant when adjusted for parity (Table 3). Multigravidity no longer differed from primigravidity concerning the risk of a positive RDT outcome when gravidity was adjusted for age. Despite the relatively few women with microscopy positive blood films and hence

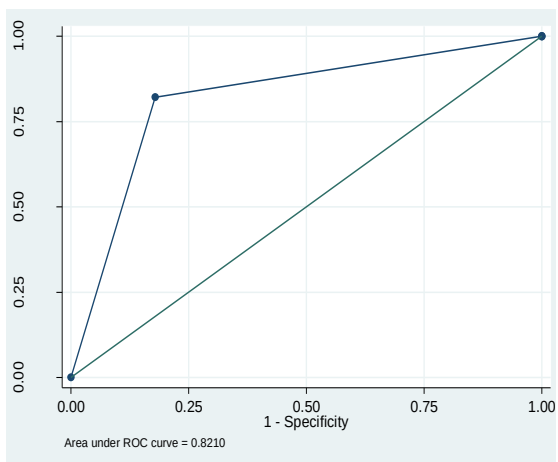

Figure 1: ROC curve for First Response® RDT result as a predictor of *P. falciparum* peripheral blood infection in asymptomatic pregnant women

Table 3: Factors assessed for association with positive First Response® rapid diagnostic tests

Variable	Crude RR		Adjusted RR	
	RR (95% CI)	*p* value	RR (95% CI)	*p* value
Age in yr.				
15-19				reference
20-24	0.64 (0.50, 0.82)	<0.001	0.71 (0.50, 0.99)	0.045
25-29	0.46 (0.35, 0.59)	<0.001	0.48 (0.32, 0.71)	<0.001
≥30	0.29 (0.22, 0.38)	<0.001	0.31 (0.19, 0.51)	<0.001
Gravidity				
primigravid				reference
Secundigravidae	0.67 (0.46, 0.99)	0.047	0.84 (0.58, 1.21)	0.354
Multigravidae	0.62 (0.44, 0.86)	0.005	0.95 (0.66, 1.38)	0.798
Parasite Density				
< 500/µL				reference
≥ 500/µL	1.97 (1.39, 2.78)	<0.001	1.00 (0.99, 1.00)	1.000
≥200/µL	2.42 (1.50, 3.93)	<0.001	1.00 (0.99, 1.00)	1.000

*RR, relative risk; CI, confidence interval; parasite density was adjusted for age and gravidity

documented parasite density, the study demonstrated a significant association between parasite density and the risk of a positive RDT result. Women with densities ≥ 500/µL were about twice as likely to record a positive RDT outcome compared to those with parasite density < 500/ µL (RR, 1.97; 95% CI: 1.39 - 2.78; $p < 0.001$). A similar result was seen for women with density ≥ 200 parasites/µL (for whom the reference population was participants with parasite density ≤ 200/uL). Adjusting for age and parity, parasite density did not affect the risk of a positive RDT outcome (Table 3). Though there was gravidity data for just about 20% (n = 307/1664) of participants, the number is considered sufficiently large to make valid inferences. Of the 17 slides corresponding to false-negative RDT results (Table 2), 5 had parasite density of 40/µL, 8 had a density of 80/µL and one each had parasite density of 120/µL, 160/µL, 240/µL and 760/µL respectively.

DISCUSSION

The study reports the performance of an HRP2-based RDT in Ghanaian pregnant women with asymptomatic peripheral blood infection using peripheral blood microscopy as a standard and emphasizes the role of other diagnostic parameters aside sensitivity and specificity in assessing the test's performance. The sensitivity (82.11%) and specificity (82.09%) were below World Health Organization recommendations of 95% sensitivity and 90% specificity and findings of ≥ 90% sensitivity in other reports [12, 24, 27] — but comparable to findings from other studies using PCR or microscopy as reference including a Burkinabe study that reported 81.5% sensitivity and PPV of 39.8% [19, 20, 28]. The sensitivity observed in the present study was higher than the 67.3% reported in a Congolese study [18] that used microscopy as a standard. The notable disparity in peripheral parasitaemia by RDT (21.57%) and microscopy (5.71%) raises concerns regarding the use of microscopy as a gold standard for assessing the diagnostic accuracy of RDTs in pregnant women. The potential for parasite sequestration into the placenta reduces the likelihood of detection in peripheral blood slides. Thus, placental sequestration may account for the low parasitaemia prevalence reported by microscopy. To mitigate this challenge, placental histology and PCR are suggested as more appropriate standards [27,29]. It is also possible rather low parasite densities did not allow detection on microscopy. Though blood film microscopy can be very sensitive with detection levels as low as 5 - 10 parasites/µL [2], several reports have shown that applying PCR can detect sub-microscopic levels of parasitaemia [30]. Also, the expertise of the microscopist and the quality of Giemsa reagent used to stain the slides may impact on the accuracy of microscopic detection of malaria parasites. However, we have confidence in the experience of the laboratory technologist and the results reported.

The study reported a PPV, the probability of having a positive blood film and by extension peripheral infection given a positive RDT test, of only 21.71%. While the RDT may appear less useful on this account, it should be remembered that the predictive values are dependent on the prevalence of the infection in the population (5.47% for *P. falciparum* and 5.71% inclusive of other species). For the same reason, the NPV is high and reflects the high prevalence of negative blood slides on microscopy. A study in Burkinabe pregnant women also showed a similar low PPV of 39.8% with a 6.0% prevalence of positive films on microscopy [28]. The likelihood ratio for a positive test was 4.58 with an upper limit of 5.28. This means a pregnant woman with peripheral *P. falciparum* infection was about 5 times more likely to have a positive RDT result than one without a peripheral infection. Conversely, a pregnant woman without a peripheral *P. falciparum* infection was 4.54-fold (n = 1/0.22) more likely to have a negative RDT result compared to another with an infection. Considering the upper limit of the 95% confidence interval for the

likelihood ratio for a negative test, a woman without infection was about 7-fold more likely to have a negative RDT result than one with a peripheral *P. falciparum* infection. Though the likelihood ratios for the positive and negative tests did not match up to the prescribed > 10 and < 0.1 needed to assert that the test has extremely useful discriminatory ability [23], the RDT showed the discriminatory ability that should be deemed useful at the population level. However, HRP2-only RDTs may not be sufficient due to HRP2 gene deletion [6-8]. A study of HRP2 diversity in Ghana [7] found that 22% and 40% of *P. falciparum* parasites assayed in Accra and Cape Coast respectively lacked the exon 2 region of the HRP2 gene and possibly the gene itself. This underlies recommendations for RDTs that detect other antigens in addition to HRP2 [9].

The ROC curve also informs on the ability of the RDT to discriminate between those who have and who do not have parasitaemia. The area under the curve is 1 for a test that discriminates perfectly and 0.5 for one with essentially no diagnostic value [31]. For the RDT used, this parameter was 0.82 and suggests moderate test accuracy. Persistence of HRP2 antigen following treatment could be a reason for some of the > 200 false-positive RDT results. One of the exclusion criteria in the trial was a history of treatment with any of the study drugs in the 2 wk preceding recruitment but it is possible that antimalarial drugs used earlier than the defined 2 wk may have resulted in HRP-2 persisting longer than 2 wk. An alternative explanation is that placental sequestration could have resulted in fewer parasites in circulation in peripheral blood that may not be detected on microscopy. About 88% (n = 15/17) of false-negative RDT results had corresponding parasite densities less than 200/µL; a finding corroborating the challenges of detecting low parasite densities among pregnant women in a study [28] conducted in Burkina Faso in which the median parasite density for positive RDTs was over 2000/µL while that for negative RDTs was 104/µL. Deletion of parasite HRP2 gene, reported in West Africa including Ghana [6-8], may underlie the false-negative RDT results but evaluating the phenomenon was outside the scope of the present study.

Women of older age groups and higher parities were less likely to show positive RDT results though the latter did not influence RDT outcome when adjusted for age. Some studies [32,33] have described higher levels of partially acquired immunity with increasing age and gravidity. This may limit high parasite densities with a reduced risk of having positive RDT outcomes. Over 80% of the women in the trial had parasite density less than 500/µL at baseline [25]. In reporting the study findings, we have sought to improve appreciation of the roles of other diagnostic parameters aside from the often-reported sensitivity and specificity in assessing malaria RDT performance. Using microscopy as a gold standard in the present study was a limitation and possibly led to false-positive RDT results from missed placental infections. However, the sensitivity reported was comparable to studies in pregnant women in which PCR alone or in combination with microscopy was the reference [18,19,24]. Another limitation was the inability to do PCR evaluation of the > 200 false-positive RDT results as this would have brought clarity to the true status of these RDT results.

Conclusion

The HRP2-only RDT used showed moderate sensitivity and specificity in detecting malaria infection in pregnant women. It also showed good likelihood ratios for both positive and negative tests and an appreciable area under the ROC curve suggesting good accuracy despite the observation of many false-positive results. However, evidence of HRP2 gene deletion limits the use of HRP2-only RDTs in general and backs the use of RDT based on combined antigens. Further research is needed to describe the burden of HRP2 gene deletion over wider areas of Ghana and on how declining malaria transmission will impact the performance of RDTs in pregnant women.

DECLARATIONS

Ethical considerations

Ethical approval for the trial was obtained from the Committee for Human Research and Publication Ethics (CHRPE), School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Ghana (CHRPE/190/10) and registered in a clinical trial database ((NCT01231113)

Consent to publish

All authors consented to the publication of the manuscript.

Funding

This work was supported by the Malaria Capacity Development Consortium which was also funded by the Wellcome Trust Grant WT084289MA and Bill & Melinda Gates Foundation Grant 51941 (http://www.mcdconsortium.org). The funding body had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Competing Interests

No conflict of interest was reported by the authors.

Author contributions

JO and HT designed the study. JO supervised data collection, analyzed the data, and wrote the initial draft. HT, MA, and PM critically revised and made major contributions to the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We are grateful to the pregnant women who participated in the trial and the study team.

Availability of data

The datasets used and/or analysed in the study are available from the corresponding author upon reasonable request.

REFERENCES

1. Kattenberg JH, Tahita CM, Versteeg IAJ, Tinto H, Traoré-Coulibaly M, Schallig HDFH, Mens PF (2012) 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 Heal 17:550–557. https://doi.org/10.1111/j.1365-3156.2012.02975.x
2. Moody A (2002) Rapid diagnostic tests for malaria parasites. Clin. Microbiol. Rev. 15:66–78
3. McMorrow ML, Masanja MI, Abdulla SMK, Kahigwa E, Kachur SP (2008) Challenges in routine implementation and quality control of rapid diagnostic tests for malaria-Rufiji District, Tanzania. Am J Trop Med Hyg 79:385–390. https://doi.org/10.4269/ajtmh.2008.79.385
4. Kumar N, Pande V, Bhatt RM, Shah NK, Mishra N, Srivastava B, Valecha N, Anvikar AR (2013) Genetic deletion of HRP2 and HRP3 in Indian Plasmodium falciparum population and false negative malaria rapid diagnostic test. Acta Trop 125:119–121. https://doi.org/10.1016/j.actatropica.2012.09.0 15
5. Baker J, Gatton ML, Peters J, Ho M-F, McCarthy JS, Cheng Q (2011) Transcription and Expression of Plasmodium falciparum Histidine-Rich Proteins in Different Stages and Strains: Implications for Rapid Diagnostic Tests. PLoS One 6(7):e22593 . https://doi.org/10.1371/journal.pone.0022593
6. Wurtz N, Fall B, Bui K, Pascual A, Fall M, Camara C, Diatta B, Fall KB, Mbaye PS, Diémé Y, Bercion R, Wade B, Briolant S, Pradines B (2013) Pfhrp2 and pfhrp3 polymorphisms in Plasmodium falciparum isolates from Dakar, Senegal: Impact on rapid malaria diagnostic tests. Malar J 12(1):1–8. https://doi.org/10.1186/1475-2875-12-34
7. Amoah LE, Abankwa J, Oppong A (2016) Plasmodium falciparum histidine rich protein-2 diversity and the implications for PfHRP 2: Based malaria rapid diagnostic tests in Ghana. Malar J 15(1):101. https://doi.org/10.1186/s12936-016-1159-z
8. Gendrot M, Fawaz R, Dormoi J, Madamet M, Pradines B (2019) Genetic diversity and deletion of Plasmodium falciparum histidine-rich protein 2 and 3: a threat to diagnosis of P. falciparum malaria. Clin. Microbiol. Infect. 25(5):580–585
9. WHO/FIND/CDC (2018) Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: round 8 (2016–2018). Geneva: World Health Organization; 2018
10. Khan WA, Galagan SR, Prue CS, Khyang J, Ahmed S, Ram M, Alam MS, Haq MZ, Akter J, Glass G, Norris DE, Shields T, Sack DA, Sullivan DJ, Nyunt MM (2014) Asymptomatic Plasmodium falciparum Malaria in Pregnant Women in the Chittagong Hill Districts of Bangladesh. PLoS One 9(5):e98442 . https://doi.org/10.1371/journal.pone.0098442
11. Carmona-Fonseca J, Arango E (2017) Asymptomatic plasmodial infection in pregnant women: A global scenario. J Vector Borne Dis 54(3):201–206 . https://doi.org/10.4103/0972-9062.217610
12. WHO/FIND/CDC (2014) WHO | Malaria rapid diagnostic test performance. Results of WHO product testing of malaria RDTs: Round 5 (2013). World Health Organization
13. Slater HC, Ross A, Ouédraogo AL, White LJ, Nguon C, Walker PGT, Ngor P, Aguas R, Silal SP, Dondorp AM, La Barre P, Burton R, Sauerwein RW, Drakeley C, Smith TA, Bousema T, Ghani AC (2015) Assessing the impact of next-generation rapid diagnostic tests on Plasmodium falciparum malaria elimination strategies. Nature 528(7580):S94–S10. https://doi.org/10.1038/nature16040
14. Steketee RW, Nahlen BL, Parise ME, Menendez C (2001) The Burden of Malaria in Pregnancy in Malaria-Endemic Areas. Am J Trop Med Hyg 64(1):28–35
15. Guyatt HL, Snow RW (2004) Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa. Clin. Microbiol. Rev. 17(4):760–769
16. Falade CO, Tongo OO, Ogunkunle OO, Orimadegun AE (2010) Effects of malaria in pregnancy on newborn anthropometry. J Infect Dev Ctries 4(7):448–453. https://doi.org/10.3855/jidc.329
17. Minja DT, Schmiegelow C, Oesterholt M, Magistrado PA, Boström S, John D, Pehrson C, Andersen D, Deloron P, Salanti A, Lemnge M, Luty AJ, Alifrangis M, Theander T, Lusingu JP (2012) Reliability of rapid diagnostic tests in diagnosing pregnancy-associated malaria in north-eastern Tanzania. Malar J 11(1):211. https://doi.org/10.1186/1475-2875-11-211
18. Matangila JR, Lufuluabo J, Ibalanky AL, Inocêncio Da Luz RA, Lutumba P, Van Geertruyden JP (2014) Asymptomatic Plasmodium falciparum infection is associated with anaemia in pregnancy and can be more cost-effectively detected by rapid diagnostic test than by microscopy in Kinshasa, Democratic Republic of the Congo. Malar J 13(1):132. https://doi.org/10.1186/1475-2875-13-132
19. Vásquez AM, Medina AC, Tobón-Castaño A, Posada M, Vélez GJ, Campillo A, González IJ, Ding X (2018) Performance of a highly sensitive rapid diagnostic test (HS-RDT) for detecting malaria in peripheral and placental blood samples from pregnant women in Colombia. PLoS One 13(8):e0201769. https://doi.org/10.1371/journal.pone.0201769
20. Williams JE, Cairns M, Njie F, Laryea Quaye S, Awine T, Oduro A, Tagbor H, Bojang K, Magnussen P, Ter Kuile FO, Woukeu A, Milligan P, Chandramohan D, Greenwood B (2016) The performance of a rapid diagnostic test in detecting malaria infection in pregnant women and the impact of missed infections. Clin Infect Dis 62(7):837–844. https://doi.org/10.1093/cid/civ1198
21. Kyabayinze DJ, Zongo I, Cunningham J, Gatton M, Angutoko P, Ategeka J, Compaoré YD, Muehlenbachs A, Mulondo J, Nakalembe M, Somé FA, Ouattara A, Rouamba N, Ouédraogo JB, Hopkins H, Bell D (2016) HRP2 and pLDH-based rapid diagnostic tests, expert microscopy, and PCR for detection of malaria infection during pregnancy and at delivery in areas of varied transmission: A prospective cohort study in Burkina Faso and Uganda. PLoS One 11(7):e0156954 . https://doi.org/10.1371/journal.pone.0156954
22. Ranganathan P, Aggarwal R (2018) Common pitfalls in statistical analysis: Understanding the properties of diagnostic tests - Part 1. Perspect Clin Res 9(1):40–43. https://doi.org/10.4103/picr.PICR_170_17
23. Ranganathan P, Aggarwal R (2018) Understanding the properties of diagnostic tests – Part 2: Likelihood ratios. Perspect Clin Res 9(2):99–102 . https://doi.org/10.4103/picr.picr_41_18
24. Anabire NG, Aryee PA, Abdul-Karim A, Abdulai IB, Quaye O, Awandare GA, Helegbe GK (2019) Prevalence of malaria and hepatitis B among pregnant women in Northern Ghana: Comparing RDTs with PCR. PLoS One 14(2):e0210365 . https://doi.org/10.1371/journal.pone.0210365
25. Osarfo J, Tagbor H, Cairns M, Alifrangis M, Magnussen P (2017) Dihydroartemisinin-piperaquine versus artesunate-amodiaquine for treatment of malaria infection in pregnancy in Ghana: an open-label, randomised, non-inferiority trial. Trop

Med Int Heal 22(8):1043–1052. https://doi.org/10.1111/tmi.12905

26. Cochran WG (1977) Determination of appropriate Sample Size: Sampling techniques (3rd ed.). In: New York: John Wiley & Sons.
27. Kattenberg JH, Ochodo EA, Boer KR, Schallig HD, Mens PF, Leeflang MM (2011) Systematic review and meta-analysis: Rapid diagnostic tests versus placental histology, microscopy and PCR for malaria in pregnant women. Malar. J. 10(1):321
28. Ruizendaal E, Schallig HDFH, Scott S, Traore-Coulibaly M, Bradley J, Lompo P, Natama HM, Traore O, Valea I, Dierickx S, Drabo KM, Pagnoni F, D'Alessandro U, Tinto H, Mens PF (2017) Evaluation of malaria screening during pregnancy with rapid diagnostic tests performed by community health workers in Burkina Faso. Am J Trop Med Hyg 97(4):1190–1197. https://doi.org/10.4269/ajtmh.17-0138
29. Conroy AL, McDonald CR, Kain KC (2012) Malaria in pregnancy: diagnosing infection and identifying fetal risk. Expert Rev. Anti. Infect. Ther. 10(11):1331–1342
30. Wu L, Van Den Hoogen LL, Slater H, Walker PGT, Ghani AC, Drakeley CJ, Okell LC (2015) Comparison of diagnostics for the detection of asymptomatic Plasmodium falciparum infections to inform control and elimination strategies. Nature 528(7580): S86–S93. https://doi.org/10.1038/nature16039
31. Aggarwal R, Ranganathan P (2018) Understanding diagnostic tests - Part 3: Receiver operating characteristic curves. Perspect Clin Res 9(3):145–148. https://doi.org/10.4103/picr.PICR_87_18
32. Fried M, Duffy PE (1996) Adherence of Plasmodium falciparum to chondroitin sulfate A in the human placenta. Science 272(5267):1502–1504. https://doi.org/10.1126/science. 272.5267.1502
33. Hviid L (2005) Naturally acquired immunity to Plasmodium falciparum malaria in Africa. Acta Trop 95(3):270–275. https://doi.org/10.1016/j.actatropica.2005.06.012