Abstract

Background: Malaria is still the primary cause of pediatric deaths. The efficient management of pediatric malaria requires its rapid and accurate diagnosis. To fulfill this requirement, rapid diagnostic tests have been developed, but their evaluation before commercialization is never exhaustive. The aim of this study was to evaluate the performance of a rapid diagnostic test (SD Bioline Malaria Antigen P.f/Pan) to diagnose malaria in children.

Materials and Methods: Testing was conducted on children aged between 6 months and 15 years who were examined at the “Centre Mère Enfant (CME) of the “Chantal Biya” Foundation (FCB), as a result of fever. Enrollment took place from April to October 2014. All children presenting with fever were sampled (3ml of blood). These blood samples were tested for malaria using microscopy on a thick blood smear and by a rapid diagnostic test (RDT) SD Bioline Malariae Antigen P.f/Pan.

Results: A total of 249 children were enrolled in this study. Malaria presence as determined by microscopy and by RDT was 30.9% and 58.2% respectively. The sensitivity, specificity, positive and negative predictive values compared to microscopy were: 75; 48.8; 39, and 81.6%. With these performances, the malaria SD Bioline rapid test presents lower values compared to WHO recommendations for rapid tests (sensitivity > 95%) in children.

Conclusion: SD Bioline Malaria Antigen P.f/Pan test should only be used in peripheral health structures that lack resources, and should be aided by clinical diagnosis.

Keyword: Malaria; Rapid diagnostic test; Performance; thick blood smear; Microscopy

Introduction

Malaria is a life-threatening blood disease caused by Plasmodium spp. and is transmitted to humans by the Anopheles mosquito. Once bitten, parasites multiply in the host's liver before infecting and destroying red blood cells. These are the exclusive vectors of these parasites with about 400 species, nearly 60 transmitting the disease (Brysker et al., 1988; Pick, 1994). The disease is particularly prevalent in tropical areas (Pick, 1994). The disease can be controlled and treated if diagnosed early. Unfortunately, this is not possible in some areas of the world lacking medical facilities, where malaria outbreaks can occur. At the moment, no malaria vaccine is available.

According to WHO estimates, 300 to 500 million cases of malaria occur each year worldwide, causing 1.5 to 2.7 million deaths. It is a scourge in the warm tropical regions of Africa, South and Central America and Asia where the disease is quasi-permanently present. In 2009, the disease was endemic in 109 countries. Those countries also happen to be among the poorest in the world- “poverty belt” (Gentillini, 1995). Forty-five (45) of these 109 countries are located in sub-Saharan Africa alone accounting for almost 90% of the total incidence of malaria (WHO, 2009a). In Cameroon, in 2010, malaria was still responsible for 40.1% of overall hospital morbidity and 40% mortality in children under 5. Two million cases are recorded in health facilities each year and malaria care and management cost represents 40% of household expenditure on health and resulted in a 1.3% loss in growth rate in 2007, according to the Ministry of Public Health (MSP). Here, over 90% of malaria cases are due to P. falciparum (MSP, 2010). The strategy for the management of malaria is modeled on WHO recommendations. Initially, diagnosis was based on clinical symptoms, including fever, even when laboratory confirmation was not done (Lalloo et al., 1992; Zurovac et al., 2006).
But this strategy showed its limitations following the emergence of vector resistance to insecticides and Plasmodium resistance to anti-malarials. The consequences were chromosomal mutations especially to chloroquine (Ononi et al., 1982) in most countries affected by *P. falciparum* including Cameroon.

In the 2006 and 2010 guidelines, WHO reviewed its strategy and recommended prompt parasitological confirmation of all clinical cases before starting treatment except where this is not possible. This requirement for laboratory confirmation is a problem because of the difficulty in making available quality microscopy (confirmatory test) and experienced personnel (Moody, 2002).

This binding requirement for quality microscopy limits access to quality care and management of malaria (Mseleem et al, 2009). In Cameroon, more than half of the reported cases are not confirmed in the laboratory and a large population trend towards self-medication has been noted (MSP, 2010). Furthermore, studies by Durrheim et al. (1997), have shown that the results of microscopic examinations differ significantly from one laboratory to another and from one technician to another. Hence, the introduction of rapid diagnostic tests (RDTs) for malaria in the early 90s by WHO was suggested as a credible alternative to conventional microscopy (Lubell et al., 2007; Wonsrichanalai et al., 2007).

To date, several malaria RDTs are available, detecting one or more antigens of the histidine-rich protein 2 (HRP-2) from *Plasmodium falciparum* and the parasite-specific lactate dehydrogenase (pLDH) or *Plasmodium* aldolase from the parasite glycolytic pathway found in all species (Moody, 2002). Many studies have been published on the worldwide assessment of RDT performance with very variable results according to manufacturers, contexts, regions and target populations. The disparity of published results for these tests says a lot about the complexity to make the choice of a RDT for malaria among the very many existing products by different firms (22 in use in Cameroon) (Ali et al., 2016). Furthermore, the performance of these tests is influenced by the genetic variability of the parasites (Baker et al., 2005).

Given that countries are increasingly willing to spend money in scaling up diagnosis tests for malaria, it is necessary to guide them in the choice of rapid diagnostic tests that meet international standards of quality and give information on the selection criteria recommended for the purchase of rapid diagnostic tests for malaria.

The objectives of this work were: to determine the performance of RDT SD Malaria Antigen Pf/Pan (SD- RDT) in the laboratory diagnosis of malaria in Yaoundé on children aged 6 months to 15 years by comparison to the thick blood smear test namely: sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

**Materials and Methods**

**Design, Setting, and Population Studied**

This study was carried out at the “Centre Mère Enfant (CME) of the “Chantal Biya” Foundation (FCB) in Yaoundé from April 2014 to October 2014. This is a reference health facility for children, located in the political capital of Cameroon (Yaoundé). It is visited by patients living in Yaoundé as well as patients referred from surrounding health facilities and even surrounding Regions.

Included in this study, were children aged 6 months to 15 years consulting in the pediatric ward of the CME-FCB and had a fever of 38°C or above (or had symptoms characteristic of malaria). A questionnaire was administered to the mother of the child and data such as age, sex, Long Lasting Insecticide-treated bed nets (LLINs) usage and HIV status were collected.

Blood was collected from each of these children and used to detect Plasmodium parasites (thick blood smear) or Plasmodium antigens (SD-RDT). Microscopy was done by two independent technicians and the results were compared. Discordant results were repeated by a third microscopist. RDT testing was done according to the manufacturer’s instructions (Standard Diagnostics Inc. 156-68, Hagal-dong Giheung-guYongin-sikyonggi-do, Korea 446/930). The SD-RDT is a rapid, qualitative and differential test for the detection of histidine-rich protein II (HRP-II) antigen of *Plasmodium falciparum* and common *Plasmodium* lactate dehydrogenase (pLDH). Its claimed sensitivity is 99.7% (P.f), 95.5% (non-P.f) and specificity of 99.5%.

**Ethics Consideration**

The study received the approval of the National Ethical Committee (N° 2013/03/086/L/ CNERSH/SP) and the authorization of the Ministry of Public Health (631-15.13). Parents or guardians gave informed and written proxy consent before enrollment.

**Statistical Analysis**

Data were recorded in Microsoft Excel 2010 and exported to IBM-SPSS Version 21 for statistical analysis. Quantitative variables were reported as number (%). Since continuous variables deviated significantly from normality, the median with the interquartile range (IQR) was used. Associations were quantified with odds ratios (OR) with 95% confidence interval (CI). The Cochran-Armitage test was used to assess the trend with ordinal variables.
The Receiver Operating Characteristic (ROC) curve was used to assess the predictive performances of SD-RDT. P-values less than 5% were considered statistically significant.

Results

A total of 249 children aged from 6 months to 15 years were recruited, with a median age of 2 years [Interquartile range: 11 months-4 years]. They were 134 (53.8%) boys and 115 (46.2%) girls, thus a sex ratio (B/G) of 1.16 in favor of boys. The overall descriptive characteristic of the study population is presented in Table 1.

Table 1: Descriptive characteristic of the study population: Microscopy results according to sex, age, bed net usage and HIV status

|                        | Microscopy Negative | Microscopy Positive | Total 249 | OR(95%CI) | P       |
|------------------------|---------------------|---------------------|-----------|-----------|---------|
| **Sex**                |                     |                     |           |           |         |
| Boys                   | 102(76.1)           | 32(23.9)            | 134(53.8) | 1         | 0.015   |
| Girls                  | 71(61.7)            | 44(38.3)            | 115(46.2) | 1.97(1.14-3.41) | 0.015   |
| **Age (years)***       |                     |                     |           |           |         |
| <=2                    | 107(79.3)           | 28(20.7)            | 135(54.2) | 1         | 0.018   |
| 2-5                    | 44(63.8)            | 25(36.2)            | 69(27.7)  | 2.17(1.14-4.13) | 0.000   |
| >=5                    | 22(48.9)            | 23(51.1)            | 45(18.1)  | 4.00(1.95-8.19) | 0.000   |
| **Bed net usage**      |                     |                     |           |           |         |
| No                     | 57(68.7)            | 26(31.3)            | 83(33.3)  | 1         | 0.85    |
| Yes                    | 116(69.9)           | 50(30.1)            | 166(66.7) | 0.94(0.53-1.67) | 0.025   |
| **HIV**                |                     |                     |           |           |         |
| Negative               | 142(66.7)           | 71(33.3)            | 213(85.5) | 3.1(1.15-8.31) | 0.025   |
| Positive               | 31(86.1)            | 5(13.9)             | 36(14.5)  | 1         |         |

* p-Cochran-Armitage test of trend = 0.00006

The prevalence of malaria infection confirmed by microscopy was 30.5% (76/249). The prevalence determined using the SD-RDT was 58.2% (145/249). Table 1 also shows that the prevalence of malaria was significantly higher in girls (38.3%) compared to boys (23.9%); girls were nearly twice as likely to be positive than boys (OR = 1.97(1.14-3.41), P = 0.015). The prevalence of malaria decreased significantly (p < 0.0001) as age increased.

Children older than 5 years and those aged 2-5 years were respectively four times (OR = 4(1.95-8.19), p = 0.001) and two times (OR = 2.17(1.14-4.13), p = 0.018) more likely to be positive than boys (OR = 1.97(1.14-3.41), P = 0.015). The prevalence of malaria decreased significantly (p < 0.0001) as age increased.

Changes in the biting pattern of the malaria vectors between biting earlier or later in the night and biting outdoors (Expert Committee on Malaria, 2000; Maxwell et al., 1998) could justify why no difference was found between LLINS usage and malaria prevalence. We found that bed net usage vary with the age as shown in Table 2.

Table 2: Bed net usage according to age

| Age (years, p-Cochran-Armitage test of trend=0.000) | No n (%) | Bet net usage | Yes n (%) | Total | OR(95%CI) | P       |
|---------------------------------------------------|----------|---------------|-----------|-------|-----------|---------|
| <=2                                               | 34(25.2) | 101(74.8)     | 135(54.2) | 4.46(2.19-9.08) | 0.00    |
| 2-5                                               | 22(31.9) | 47(68.1)      | 69(27.7)  | 3.20(1.47-7.00) | 0.004   |
| >=5                                               | 27(60.0) | 18(40.0)      | 45(18.1)  | 1     |           |         |
The prevalence of HIV was 14.5% (36/249); malaria prevalence was higher among HIV negative children. In fact, HIV negative children were three time more likely to have malaria than HIV infected ones (OR = 3.1(1.15-8.31), P = 0.015). There were 5 children infected both with malaria and HIV, thus a co-infection rate of 2% (5/249). According to Table 3, the number of false positives recorded for SD-RDT was 88 and that of false negatives 19. The sensitivity and specificity of SD-RDT were respectively 75% (57/76) and 49.1% (85/173). The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were respectively 39.3% (57/145) and 81.7% (85/104).

| Table 3: Performance of the SD Bioline-TDR compared to microscopy |
|-------------------|-----------------|-----------------|-----------------|
| SD Bioline-TDR    | Microscopy      | Negative        | positive        | Total |
|                   |                 | 85 TN           | 19 FN           | 104   |
|                   |                 | 88 FP           | 57 TP           | 145   |
| Total             |                 | 173             | 76              | 249   |

*TN: True Negative; FP: False Positive; FN: False Negative; TP: True positive*

The correlation between the parasitaemia and the result of the RDT test was also analyzed (Table 4).

| Table 4: SD Bioline-TDR results according parasitemia (number of trophozoites/µl) |
|---------------------------------|----------|----------|----------|
| Positive n (%)                  | 76(30.5) | 100(39.0)| 249(100) |
| Negative n (%)                  | 173(69.5)| 70(29.5)| 243(97)  |
| Parasitemia level               | ≤50      | [50,100]| [100,500]| [500,1000]| [1000,5000]| >5000 |
| 1(50.0)                         | 1(50.0) | 1(50.0) | 2(1.8)   | 2(1.8)   | 2(1.8)   |
| 1(25.0)                         | 3(75.0) | 8(27.3) | 4(100)   | 4(100)   | 4(100)   |
| 3(27.3)                         | 8(72.7) | 11(14.4)| 11(14.4) | 11(14.4) | 11(14.4) |
| 0(0.0)                          | 4(100)  | 4(100)  | 4(100)   | 4(100)   | 4(100)   |
| 2(9.1)                          | 20(90.9)| 22(28.9)| 22(28.9) | 22(28.9) | 22(28.9) |
| 12(36.4)                        | 21(63.6)| 33(43.4)| 33(43.4) | 33(43.4) | 33(43.4) |

In particular, Figure 1 shows that the efficacy of the RDT increases with the level of parasitaemia and reaches a maximum of 100% with 500-1000 trophozoites/µl, after which it decreased to 63.6% when more than 5,000 trophozoites/µl were present.

![Figure 1](image)

*Figure 1: Prevalence (%) of malaria using SD Bioline Malaria Ag P.f/Pan-TDR according to parasitemia level (number of trophozoites/µl).*

The ROC curve for SD-RDT with parasitaemia (Figure 2) shows a low area under the curve of 0.446 (95% CI = 0.29-0.60). This means that because of the low sensitivity and specificity of the SD-RDT when compared with microscopy, the RDT is not very suitable for diagnosis alone.
Figure 2: ROC Curve for SD Bioline Malaria Ag P.f/Pan -TDR with parasitemia

We compared the results obtained in our study with those of other authors. These results are summarized in Table 5.

Table 5: Performance evaluation of SD bioline malaria Ag pf/pan RDT against Microscopy by various authors

| Authors        | Country        | Age range study population | Se  | Sp   | Reference                                      |
|----------------|----------------|----------------------------|-----|------|-----------------------------------------------|
| Ratsimbasoa et al | Madagascar    | 0.5-63                     | 92.9| 98.9 | AJTMH, 2008                                   |
| Ogouyemi et al  | Benin          | 0.5-70                      | 96.3| 95.6 | Bulletin de la société pathologie exotique, 2013 |
| Djalle et al    | Central African Republic | All age               | 88.2| /    | BMC infect Dis, 2014                           |
| Akotet et al    | Gabon          | 1-8                        | 96.8| 89.3 | Malar chemotherapy control and elimination, 2014  |
| Tseroni et al   | Greece         | 3-67                       | 97.4| 99.4 | PlosOne, 2015                                 |
| Sakthivel et al | India          | 1-60                       | 78.3| 81.8 | Am J Epidemiol Inf Dis, 2015                   |
| Tadesse et al   | Ethiopia       | 1-80                       | 99.5| 98.0 | Rev Inst Med Trop Sao Paulo, 2016              |
| Ali et al       | Cameroon       | 1-16                       | 98.0| 65.0 | Malar J, 2016                                 |

Se: Sensitivity, Sp: Specificity

Discussion

The sensitivity of the SD BIOLINE Malariae Antigen P.f / Pan was 75% in this study, which is less than that recommended by WHO for a RDT (> 95%). The sensitivity of this RDT found in several studies varies, as shown in Table 5, from 88.2 to 99.5%. These studies were done in several countries, endemic and non endemic for malaria, on several geographic populations of different age range and with various parasitaemia levels and Plasmodium species. The difference in sensitivity obtained in these studies could be explained by the genetic polymorphism in HRP2 (Baker et al., 2005; Kilian et al., 1997; Amos 2005). Anti-HRP2 antibodies produced in some patients leading to the neutralization of HRP2 produced by plasmodia could also be the reason for the poor sensitivity (Biswas et al., 2005; Lee et al., 2006). Low parasitaemia levels could also explain the various sensitivities of this RDT, but they do not seem to be the case in our study population, as 75% of the patients positive for RDT had a parasitic load > 501/μL. In a country like Cameroon with regular exposure to Plasmodium one observes tolerance (clinically asymptomatic); requiring a higher rate of blood parasites for positive RDT (Munier et al., 2009).

Furthermore, deletions or mutations in the HRP 2 gene may reduce the sensitivity of these RDTs (Baker et al., 2005; Kumar et al., 2013; Wurtz et al., 2013; Maltha et al., 2012; Koita et al., 2012; Houze et al., 2011). The specificity of the SD BIOLINE Malariae Antigen Pf/pan obtained in this study was 49.1%. Other studies have shown a specificity ranging from 65-99.4% (Table 5).

The relatively high rate of false positives could be due to several factors such as the presence of jaundice in patients (Grobusch et al., 1999); rheumatoid antigens (Bartoloni et al., 1998; Iqbal et al., 2000) or other antibodies (WHO, 2011; Gillet et al., 2013); to the persistence of HRP2 antigen in the blood several days after clearance of
Plasmodium (Mayxay et al., 2001; Wonsrichanalai et al., 2007; Rakotonirina et al., 2008; WHO, 2008 b) or by self-medication taken before deciding to consult when the symptoms persist. All these possibilities could explain the high rate of false positive obtained with SD-RDT in all age groups.

The Negative Predictive Value (NPV) which is the probability that a patient does not have malaria when the SD-RDT is negative, was 81.6% (Table 3) This indicates that out of 100 patients testing negative with using the SD-RDT, about 82 were actually free from malaria (negative microscopy) leading to 18 patients being wrongly prescribed anti-malarial drugs. If this can be avoided then it could contribute to reducing the phenomenon of resistance in areas of high transmission (Molyneux et al., 1999; Hastings et al., 2000), responsible for treatment failure.

The positive predictive value (PPV), measures the probability that a patient tested positive by SD-RDT is actually infected with Plasmodium. In this study PPV was 39.3%, lower than 86.7% obtained in Benin (Ogouyènê-Hounto et al., 2013). This means that, 60 times out of 100, a person whose SD-RDT was positive had no malaria (when tested by microscopy). This will lead to over-prescription, development of resistance and money wasted (Grawley et al., 2010). Hence, it is important to use two diagnostic tests for better decision making, just like in other cases such as HIV testing that combines 2-3 tests, before deciding that drug treatment is appropriate.

The predictive values (PPV and NPV) always depend on the prevalence of the disease in the area of study. Indeed, for a test with given sensitivity and specificity, predictive values (PPV and NPV) differ with disease prevalence in the study population (Mac Morrow, 2008).

Ali et al. (2016) evaluated the accuracy of 22 RDT in Cameroon on children, among which SD Bioline Malaria Antigen P. f./Pan was included. These 22 tests have sensitivity ranging from 63% to 98%; and a specificity ranging from 48% to 92%. We thus suggest that as in HIV diagnosis, an algorithm should be developed to use two tests, one with higher sensitivity and the second with higher specificity.

We have observed the prozone effect with SD-RDT as shown in Figure 1 and by other authors (Gillet et al., 2009b). Maximum sensitivity was at a parasitaemia level between 500-1000 trophozoites/µl. This presents a major limitation of this RDT as parasitaemia is usually high in children.

Although HIV prevalence in our study population was 14.5%, malaria-HIV co-infection was 2%. Bed net usage decreased linearly as the age group increased (p-Cochran-Armitage test of trend = 0.000); starting from 74.8% for less than 2 years, 68.1% for those aged 2-5 years and 40% for 5 years and above (Table 2). Children under 2 years and those aged 2-5 years were respectively four times (OR = 4.46(2.19-9.08), p = 0.00) and three times (OR = 3.20(1.47-7.00), p = 0.004) more likely to use a bed net than those who were 5 years and older. As the child grows, the usage of bed nets becomes problematic, as they could easily get out of the bed net and be exposed to malaria. The prevalence of malaria was significantly higher in girls than boys with p value of 0.015. No other study on pediatric malaria has shown gender susceptibility (EDS-MICS, 2011). It has been shown for other infections that males are in general more susceptible to infectious diseases than females, and this has been mostly ascribed to the immunosuppressive activity of testosterone. But in this study, contradictory results were obtained. No other studies have linked the female gender to the susceptibility of malaria. This will be investigated further.

Conclusion

RDT tests are a good tool to overcome drawbacks presented by conventional diagnostic tool. But these tools should be evaluated and validated for the specific context in which they are to be used. In case this is not possible, the use of RDT for malaria should be associated with clinical symptoms. We suggest that an algorithm should be developed for malaria as is the case for RDT for HIV. We also suggest that usage of bed net be adapted according to the age of children to ensure good usage.

Declarations

Competing interests: The authors declare that they have no competing interests.

Funding: This study was funded by the Institution the “Chantal Biya” International Reference Centre-CBIRC

Acknowledgements

The study participants are acknowledged for their collaboration. We also thank Sir Richard Roberts for reading this manuscript.

References

1. Ali, I.M., Bigoga, J.D., Forsah, D.A., Cho-Ngwa, F., Tchinda, V., Moor, V.A., Fagako, J., Nyongalema, P., Nkoa, T., Same-Ekobo, A., Mbode, J., Fondjo, E., Mbcham, W.F. and Leke, R.G.F. (2016). Field evaluation of the 22 rapid diagnostic tests for community management of malaria with artesiminin combination therapy in Cameroon. Malar J., 15:31 DOI 10.1186/s12936-016-1085-0.
2. Amos, F. (2005). Performances diagnostiques du test rapide Optimal-IT: Place de la Biologie moléculaire dans l'évaluation du polymorphisme génétique du lactate déshydrogénase (LDH) de P. falciparum. Thèse de doctorat en pharmacie, Université de Bamako, Mali; 144p.

3. Akotet, B.M.K., Nkare, C.A., Mbouoronde, O.C., Mawili-Mbounba, D.P. (2014). Performances of SD Bioline Malaria Ag-PF/Pan RDT for the Diagnosis of Malaria in Febrile Patients Living In Gabon, Central Africa. Malar Chemot Cont Elimination 3: 125. doi: 10.4172/2090-2778.1000125.

4. Baker, J., McCarthy, J., Gatton, M., Kyle, D.E., Belizario, V., Luchavez, J., Bell, D., Cheng, Q. (2005). Genetic diversity of Plasmodium falciparum histidine-rich protein 2 (PfHRP2) and its effect on the performance of PfHRP2-based rapid diagnostic tests. J Infect Dis., 192:870–877.

5. Bartoloni, A., Sabatinelli, G., Benucci, M. (1998). Performance of two rapid tests for Plasmodium falciparum malaria in patients with rheumatoid factors. N Engl J Med., 338:1075.

6. Biswas, S., Tomar, D., Rao, D.N. (2005). Investigation of the kinetics of histidine-rich protein 2 and of the antibody responses to this antigen, in a group of malaria patients from India. Ann Trop Med Parasitol., 99: 553-562.

7. Bruce-Chwatt, L.J. (1952). Malaria in African infants in Nigeria. Ann. Trop. Med. Parasitol., 46:173-200.

8. Brysker, A. and Labro, M.T. (1988). paludisme et médicament. Edition Arnette. Pp. 36-65. Paris. Coleman.

9. Durrheim, D.N., Becker, P.J., Billinghamurst, K. and Brink, A. (1997). Diagnostic disagreement - the lessons learnt from malaria diagnosis in Mpmalanga. S Afr Med J., 87: 609-611.

10. Djallé, D., Gody, J.C., Moyen, J.M., Tekpa, D., O.'hedico, C., and Von Sonnenburg, F. (1997). The diagnosis of malaria: traditional and contemporary approaches. In : Médecine Tropicale. Flammarion Médecine Science, Paris., 91-122.

11. EDS-MICS (2011). Note de présentation des résultats préliminaires www.statistics-cameroon.org/.../EDS-MIC511/EDS-MIC54 Note de presentation_14 Assessed 23 dec 2016.

12. Expert Committee on Malaria. Technical Report Series – 892 (20th Report) Geneva, Switzerland: World Health Organization; 2000.

13. Gentillin, M. (1995). Maladies Parasitaires :Le Paludisme. In : Médecine Tropicale. Flammarion Médecine Science, Paris., 91-122.

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

15. Gillet, P., MumbaNgoyi, D., Lukuka, A., Kande, V., Atua, B., van Griensven, J., Muyembe, J.J., Jacobs, J., Lejon, V. (2013b). False positivity of non-targeted infections in malaria rapid diagnostic tests: the case of human African trypanosomiasis. PLoS Negl Trop Dis., 7(4): e2180.

16. Grawley, J., Chu, C., Moeve, G. and Nosten, F. (2010). Malaria in children. Lancet., 375:1468-86.

17. Grobusch, M.P., Jelinek, T., Hänscheid, T. (1999). False Positivity of Rapid Antigen Detection Tests for Diagnosis of Plasmodium falciparum Malaria: Issue Appears to Be More Complicated than Presented. J Clin Microbiol.,37(11): 3781–3782.

18. Hastings, I.M. and D'Alessandro, U. (2000). Modelling a predictable disaster: the rise and spread of drug-resistant malaria. Parasitol Today.,16: 340-7.

19. Houze, S., Hubert, V., Le Pessec, G., Le Bras, J., Clain, J. (2011). Combined deletions of pfhrp2 and pfhrp3 genes result in Plasmodium falciparum malaria false-negative rapid diagnostic test. J Clin Microbiol., 49:2694-2696.

20. Iqbal, J., Sher, A., Rab, A. (2000). Plasmodium falciparum Histidine-Rich Protein 2-Based Immunocapture Diagnostic Assay for Malaria: Cross-Reactivity with Rheumatoid Factors. J Clin Microbiol., 38:1184-1186.

21. Kilian, A., Mughus, B., Kabagambe, and Von Sonnenburg, F. (1997). Comparison of two rapid HPR-2 based diagnostic tests for P. falciparum. Trans R Soc Trop Med Hyg., 91:666-667.

22. Koita, O.A., Doumbo, O.K., Ouattara, A., Tall, L.K., Konare, A., Diakite, M., Diallo, M., Sagara, I., Masinde, G.L., Doumbo, S.N., Dolo, A., Tounkara, A., Traoré, I. and Krogstad, D.J. (2012). False-negative rapid diagnostic tests for malaria and deletion of the histidine-rich repeat region of the hrp2 gene. Am J Trop Med Hyg., 86:194-198.

23. Kumar, N., Pande, V., Bhatt, R.M., Shah, N.K., Mishra, N., Srivastava, B., Valecha, N. and Anvikar, A.R. (2013). Genetic deletion of HRP2 and HRP3 in Indian Plasmodium falciparum population and false negative malaria rapid diagnostic test. Acta Trop., 125:119–121.

24. Lalloo, D. and Naraqi, S. (1992). The diagnosis of malaria: traditional and contemporary approaches. P N G Med J., 35: 243-248.

25. Lee, N., Baker, J., Andrews, K.T. (2006). Effect of sequence variation in Plasmodium falciparum histidine-rich protein 2 on binding of specific monoclonal antibodies: implications for rapid diagnostic tests for malaria. J. Clin Microbiol., 44: 2773-8.

26. Lubell, Y., Reyburn, H., Mbakilwa, H., Mwangi, R., Chonya, B., Whitty, J.M. and Mills, A. (2007). The cost-effectiveness of parasitological Diagnost of Malaria for Malaria-suspected patients in an Era of combination therapy. Am. J. Trop. Med. Hyg.,77(suppl 6):128-132.
27. Mac Morrow, M. (2008). Challenges in routine implementation and quality control of rapid diagnostic test for malaria, Rufiji District, Tanzania. Am. J. Trop. Med. Hyg., 79(3):385-390.

28. Malthe, J., Gamboa, D., Bendezu, J., Sanchez, L., Cnops, L., Gillet, P., Jacobs, J. (2012). Rapid diagnostic tests for malaria diagnosis in the Peruvian Amazon: impact of pfhrp2 gene deletions and cross-reactions. PLoS One, 7:e43094.

29. Maxwell, C.A., Wakkibara, J., Tho, S., Curtis, C.F. (1998). Malaria-infective biting at different hours of the night. Med Vet Entomol., 12:325–327. doi: 10.1046/j.1365-2915.

30. Mayxay, M., Pukrittaya kamee, S., Chotivanich, K., Looareesuwan, S., White, N.J. (2001). Persistence of Plasmodium falciparum HRP-2 in successfully treated acute falciparum malaria. Trans R Soc Trop Med Hyg., 95:179–182.

31. Molyneux, D.H., Floyd, K., Barnish, G. and Fevre, E.M. (1999). Transmission control and drug resistance in malaria: a crucial interaction. Parasitol Today., 15: 238-40.

32. Moody, A. (2002). Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev.,15: 66–78. doi: 10.1128/CMR.15.1.66-78.2002.

33. Msellem, M.I., Martensson, A., Rotllant, G., Bhattarai, A., Stromberg, J., Kahigwa, E., Garcia, Petzold, M., Olumese, P., Ali, A. and Björkman, A. (2009). Influence of Rapid Malaria Diagnostic Tests on Treatment and Health Outcome in Fever Patients Zanzibar-A Cross over Validation Study. PLoS Med., 6(4): e1000070. https://doi.org/10.1371/journal.pmed.1000070.

34. MSP (2010). Ministère de la Santé Publique du Cameroun. Directives pour la gestion et l'utilisation rationnelle des combinaisons thérapeutiques à base d’artémisinine (ACT) au Cameroun, Mars 2010; 40p.

35. Munier, A., Diallo, A., Sokhna, C. and Chippaux, J.P. (2009). Evaluation d’un test de diagnostic rapide du paludisme dans les postes de santé rurals au Sénégal. Med Trop., 69: 496-500.

36. Ogouyémi-Hounto, A., Kinde-Gazard, D., Keke, C., Gonçalves, E., Alapini, N., Adjovi, F., Adisso, L., Bossou, C., Denon, Y.V; and Massougbodji, A. (2013). Assessment of a rapid diagnostic test and portable fluorescent microscopy for malaria diagnosis in Cotonou (Bénin). Bull. Soc. Pathol. Exot., 106: 27. doi:10.1016/j.spa.2013.01.0264-7.

37. Ononi, E., Payne, D., Grab, B., Horst, H.L., Ameida, F.J. and Joria, H. (1982). Incipient resistance of falciparum to chloroquine among a semi-immune population of the United Republic of Tanzania.I. Bull. org. Mond. Sânté., 60, 77-87.

38. Pick, A. (1994). Un Lourd tribut. Actua palu-safoni vinhrop A.M.O. 6, 1-7.

39. Rakotonirina, H., Barnadas, C., Raherigafy, R., Randrianasolo, L., Jahevitra, M., Andrianantoandro, V. and Médnard, D. (2008). Accuracy and Reliability of Malaria Diagnostic techniques for Guiding febrile outpatient treatment in Malaria-endemic countries. Am. J. Trop. Med. Hyg., 578 (2):217-221.

40. Ratsimbasoa, A., Fanazava, L., Radrianjafo, R., Ramlilijaona, J., Rafanomezantoa, H., and Ménard, D. (2008). Short Report: Evaluation of Two New Immunochromatographic Assays for Diagnosis of Malaria. Am. J. Trop. Med. Hyg., 79(5), 670–672.

41. Sakhivel, A., Sahu, S.S. (2015). Field Performance of Wondfo and SD Bioline Malaria Pf/Pan Rapid Diagnostic Tests for Malaria Diagnosis in Koraput District, Odisha State, India. Am J Epidemiol Infect Dis.,3 (2) 21-27.

42. Sehgal, V.M., Siddiqui, W.A. and Alpers, M.P. (1989). Study to evaluate the role of passive maternal immunity. Trans. R. Soc. Trop. Med. Hyg., 83:105-106.

43. Tadesse, E., Workalemahu, B. and Shimelis, T. (2016). Diagnostic performance evaluation of the sd bioline malaria antigen ag pf/pan test (05fk60) in a malaria endemic area of southern Ethiopia. Rev. Inst. Med. Trop. Sao Paulo; 58:59 http://dx.doi.org/10.1590/S1678-9946201658059.

44. Tseroni, M., Pervanioud, D., Tserkezou, P., Rachiotis, G., Pinaka, O., Baka, A. (2015). Field Application of SD Bioline Malaria Ag Pf/Pn Rapid Diagnostic Test for Malaria in Greece. PLoS ONE 10(3): e0120367. doi:10.1371/journal.pone.0120367.

45. WHO (2009a). World Malaria Report. Global Malaria Program: WHO Library Catalogue in Publications data. Geneva. 2009 : 78 P.

46. WHO (2008b). Malaria Rapid Diagnostic Test performance. Result of WHO product testing of malaria: Round 1.2008.

47. World Health Organization (2011). Malaria rapid diagnostic test performance results of WHO product testing of malaria RDTs: round 3 (2010–2011). Geneva: World Health Organization. 124 p.

48. Wonsirchanalai, C., Mazie, J., Barcus, Muth, S., Sutamihardja, and Werndorfer (2007). Microscopy and Rapid Diagnostic Test (RDT). Am. J. Trop. Med. Hyg., 77 (6suppl):119-127.

49. Wurtz, N., Fall, B., Kui, K., Pascual, A., Fall, M., Camara, C., Diatta, B., Fall, K.B., Mbaye, P.S., Dieme, 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: 34.

50. Zurovac, D., Midia, B., Ochola, S.A., English, M., Snow, R.W. (2006). Microscopy and outpatient malaria care management among older children and adults in Kenya. Trop Med Int Health., 11: 432-440.