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Diagnostic Usefulness of Five Malaria Rapid Diagnostic Kits Compared with Stained Blood Smear Microscopy

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Abstract

Malaria is a major health challenge. The causative organisms are the plasmodium parasites which are transmitted to humans via infected female anopheles' mosquitos. The gold standard for its diagnosis is light microscopy of stained blood films. This work determined the suitability of five types of malaria rapid diagnostic test (mRDT) kits; Carestart, First Response, SD Bioline, Meriscreen and NxTek as alternatives to microscopy. About 2ml venous blood was collected from each of 615 patients who showed symptoms of malaria. The blood samples were treated with the mRDKs and by microscopy using Giemsa, Leishman and Field's stains. The results revealed that 535 (87%) had plasmodium parasites by light microscopy, while the five RDTs showed varying results. The percentage sensitivity, specificity, positive predictive values and negative predictive values were RDK 1 (89.5, 87.2, 97.9, 55.4), RDK 2 (92.6, 89.5, 98.3, 64.3), RDK 3 (90.1, 82.2, 97.1, 55.3), RDK 4 (89.1, 85.2, 97.6, 53.8) and RDK 5 (92.9, 83.0, 97.3, 63.6). The mean values were 90.8, 85.4, 97.7 and 58.1 respectively at 95% confidence level. False negative results in the RDTs were observed in low parasiteamia while false positive results were observed in some patients on medication, perhaps because of the presence of the malaria antigens after the parasites had been cleared from the blood stream. mRDKs are important alternatives to microscopy only in cases of emergencies and where facilities and trained personnel are not available. The results confirm the superiority of microscopy of stained smears to mRDKs.

Keywords: Malaria, mRDK, Microscopy, Stained smears, Anopheles mosquito

1.0 Introduction

There were an estimated 247 million cases of malaria in 2021. An estimated 619 thousand cases resulted in death. More than 95 % of the cases were in Africa. Children were mostly affected and accounted for about 80 % of the deaths. Worldwide, Nigeria accounts for 31.3 % of the cases, while the Democratic Republic of the Congo accounts for 12.6 % of the cases.[1] The WHO and its partners have a target to reduce the incidence of malaria to 75 % by 2025, and about 90 % by 2030. The main focus is on pregnant women, infants, children, adolescents and mothers.[2] Early diagnosis followed by prompt treatment of malaria is a major factor in the reduction of the incidence and mortality of the disease. Prompt diagnosis also helps to reduce transmission of the parasites and unnecessary use of the artemisinin-based combination therapies. The WHO recommendation was that all suspected cases of malaria should be confirmed before administration of drugs. Malaria Rapid Diagnostic Test (mRDTs) or microscopy are recommended for the diagnosis of malaria. Microscopy method for the diagnosis of malaria, although is the gold standard is cumbersome, time consuming, requires the availability of a trained staff as well as relevant equipment. The use of mRDKs does not require the aforementioned. Therefore, they are useful alternatives where microscopy is not available. However, mRDTs may not be useful when the parasite density is low or in some uncommon species of plasmodium such as ovale and malariae.[3] Malaria is a major killer disease in many tropical countries.[4] Plasmodium falciparum is the most frequent and most pathogenic of the Plasmodium species[2], despite the many treatment options available.[5,6] WHO has recommended that the diagnosis of malaria should be made before treatment except for children in high prevalence areas where delay in treatment may be fatal.[7] Therefore, accurate test results should be available without delay. Microscopic identification of parasites in stained smears is time consuming and requires special training, although it remains the gold standard in the diagnosis of malaria.[8] mRDKs are immunochromatographic diagnostic tools that can provide results within 20 minutes. They are commercial kits and their usage does not require special training to perform and interpret the results. The gold standard for laboratory diagnosis of malaria is the microscopic examination of stained thick blood films with any of the Romanowsky dyes such as Field, Giemsa and Wright's stain.[9] Thick blood films enhance sensitivity of the microscopic technique and enables detection of very low levels of the parasites in blood. The sensitivity of thick blood films has been estimated to be equivalent to 0.001% of infected red blood cells. However, most diagnostic laboratories achieve a lower sensitivity of about 0.01 % infected red blood cells [10] due to the level of expertise.[9] The sensitivity of thin blood films has been estimated to be 1/10 when compared with thick blood films.[9] The WHO recommendation on RDT was that the results should be as accurate as possible and comparable with results derived from microscopy.[11] Their sensitivity should be above 95% when compared with microscopy of stained slides. The RDT should be able to distinguish viable parasites from products of parasites and nucleic acids from other sources not associated with viable malaria parasites.

2.0 Materials and methods

2.1 Study Design

A cross-sectional study was conducted on consented people above 18 years who presented with symptoms of malaria in Southern Nigeria. The study was from January 2023 to December 2024.

2.2 Ethical consideration

Ethical approval was obtained from the Ethics Committee of the Faculty of Science, Delta State University, Nigeria. Consent was also obtained from individuals who took part in the work.

2.3 Collection of Blood and Laboratory Analysis

A total of 615 individuals who presented with symptoms of malaria participated in this study. About 2 ml venous blood samples were collected into EDTA containers and into plain Khan tubes.[12] Thick blood films were made

from the EDTA blood samples immediately after collection of blood. Thick blood films were stained by the Giemsa's, and by the Field's staining techniques. The thin blood films were stained by the Lishman's technique. RDTs were performed on the whole blood specimens using CareStart Malaria HRP2 Pf (Access Bio), Meriscreen Malaria Pf HRP-II Ag (Meril Diagnostics), First Response Malaria Antigen Pf (HRP2) Card Test (Premier Medical Corporation), Standard Q Malaria Pf Ag Test (SD Biosensor), NxTek (Abbott) in no particular order. The results of the RDTs were reported as positive or negative.

The mRDKs use lateral flow immunoassay technology. Malaria parasites release antigens into the bloodstream which are detected by the mRDKs using the antibodies embedded in the test strip. A drop of blood is placed on the strip, and if parasite antigens are present, they bind to antibodies already coated with a dye or gold particles to produce a colored line.

2.4 Interpretation

A line referred to as the 'Control' is present to confirm the viability of the RDK. A second line indicates the presence of malaria antigens. Absence of the second line indicates no detectable malaria parasite infection.

2.5 Target antigens

Histidine-rich protein 2 (HRP2), specific to Plasmodium falciparum.
Plasmodium lactate dehydrogenase (pLDH) is present in all malaria species.

2.6 Analysis

Data were analyzed according to the Bayes' theorem. The sensitivity, specificity and predictive values were determined using microscopy as the standard.

3.0 Results

Table 1: Comparison of five malaria mRDKs to microscopy of stained smears

mRDK manufacturer	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value	Negative predictive value
1	89.5	87.2	97.9	55.4
2	92.6	89.5	98.3	64.3
3	90.1	82.2	97.1	55.3
4	89.1	85.2	97.6	53.8
5	92.9	83.0	97.3	63.6
Mean	90.8	85.4	97.7	58.1

mRDK: Rapid diagnostic kit, CI: confidence interval

Percentage mean sensitivity, specificity, positive predictive values and negative predictive values of 5 the RDTs studied compared with microscopy of stained smears for malaria parasites at a prevalence of 87% and at a confidence index of 95% shows a high sensitivity

4.0 Discussion

The names of the manufacturers of the mRDKs are replaced with numbers to prevent undue advertisement of a kit over the others. A total of 615 individuals living in malaria endemic areas and who presented with symptoms of malaria participated in this study. Of this number, 535 (87 %) had malaria parasites in their blood samples by the light microscopy method. The gold standard for the identification of malaria parasites in blood is the microscopic examination of stained thick smears of blood. This method can detect as low as 50 parasites/l (0.001 % parasitemia) compared to the overall sensitivity of 90.8% observed with the RDTs in this experiment. Microscopy can also be used to identify the species of Plasmodium to the level of 98 % when performed by well trained personnel compared with the overall specificity of 85.4% in the RDTs used in this experiment. However, microscopy has some disadvantages such as difficult and time-consuming procedures which require special training of personnel. Attempts to overcome these challenges have led to the development of several laboratory techniques for the diagnosis of malaria. These methods include fluorescence microscopy which has an improved sensitivity but without specificity. PCR would have been an excellent technique because of its sensitivity and specificity as it can detect parasites as low as 5 parasites/L but it is expensive, time consuming and requires an expert to perform it [13] and most patients cannot afford the cost of diagnosis. Immunochromatographic dipsticks are not time consuming, not microscopic and do not require special training before the test can be performed and interpreted. However, the WHO document on New Perspectives for Malaria Diagnosis states that the sensitivity for RDT remains a challenge. The usefulness of the RDTs has been emphasized in the monitoring of patients during treatment.[14,15] Another report suggested that the presence of HRP-2 antigens in blood after treatment makes this technique unreliable for monitoring of response to therapy. RDTs offer an opportunity for patients with malaria to be diagnosed instantly and in the comfort of their homes. False-negative results from RDT can be due to operator error, improper storage conditions [16], poor performance of some brands and parasite density below the limit of detection of the RDT which in most cases is about 200 parasites/μL.[17,18] The later explains the reason for the 90.8 % sensitivity in this work. Most common PfHRP2-based RDTs do not detect non-P. Falciparum parasites.[19] They detect P. falciparum histidine-rich protein 2 (PfHRP2), which is a P. falciparum-specific antigen.[20] The sensitivity observed in this work may be below the actual values because false-positive results could be as a result of the presence of PfHRP2 antigen in the blood after the parasites have been cleared from the blood by drugs because HRP2 can be detected in blood long after the parasites are cleared from the blood. This misdiagnosis is a major challenge in the use of malaria RDK because it can lead to overtreatment.[21] Lot-to-lot variations have been observed to influence performance quality of some RDT.[22] Deletion of pfhrp2/3 genes have recently been observed in some parasites. These have been shown to cause false negative results.[23] because PfHRP2-based RDTs [24], will not detect the parasites. [25,26,27]. False negative results arising from the use of malaria RDT were observed in this study when compared with microscopy. Previous authors have also reported extensive and varying geographical variations in the results from RDTs. These explain the predictive values seen in this study. The differences have contributed to difficulties in the control efforts of malaria in some areas.

5.0 Conclusion

Malaria rapid diagnostic kits (mRDTs) provide reasonable sensitivity and specificity but, they cannot be completely relied upon because of their false negatives in cases of low parasitaemia and false positives in patients with residual antigens when active parasites are no longer in their blood stream. Although, mRDTs are useful in emergencies and in resource-limited settings where microscopy is unavailable, light microscopy of stained blood smears remains the superior and most reliable diagnostic method in the diagnosis of malaria.

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7.0 Financial support and sponsorship

Nil.

8.0 Conflicts of interest

There are no conflicts of interest.

References

1. World Health Organization. (2021). World malaria report 2021. World Health Organization.
2. Kho, S., Anstey, N. M., Barber, B. E., William, T., Rajahram, G. S., & Yeo, T. W. (2022). Diagnostic performance of a 5-plex malaria immunoassay in regions co-endemic for Plasmodium falciparum, P. vivax, P. knowlesi, P. malariae, and P. ovale. Scientific Reports, 12, Article 7286. https://doi.org/10.1038/s41598-022-11286-1
3. Bini, N. E., Magdaline, D. E., Avwioro, O. G., & Oforofuo, I. A. O. (2012). Glucose-6-phosphate dehydrogenase activity in jaundiced and non-jaundiced children with malaria parasitaemia in Delta State, Nigeria. Continental Journal of Biomedical Sciences, 6(1), 25–30.
4. Avwioro, O. G., Iyiola, S., & Enoghayin, E. E. (2010). Effects of some Nigerian antimalarial medicinal plants on glucose levels in Wistar rats. Archives of Applied Science Research, 2(3), 112–116.
5. Avwioro, O. G. (2010). Effectiveness of some medicinal plant decoctions in the treatment of malaria in Nigeria. Annals of Biological Research, 1(2), 230–237.
6. World Health Organization. (2006). The role of laboratory diagnosis to support malaria disease management: Focus on the use of rapid diagnostic tests in areas of high transmission. World Health Organization.
7. Biruksew, A., Demeke, D., Birhanu, Z., Kebede, E., Golassa, L., Mathebula, E. M., & Yewhalaw, D. (2023). Diagnostic performance of NxTek™ Eliminate Malaria-Pf test for the detection of Plasmodium falciparum in school children with asymptomatic malaria. Malaria Journal, 22, Article 112. https://doi.org/10.1186/s12936-023-04529-y
8. Warhurst, D. C., & Williams, J. E. (1996). Laboratory diagnosis of malaria. Journal of Clinical Pathology, 49(7), 533–538.
9. Milne, L. M., Chiodini, P. L., & Warhurst, D. C. (1994). Accuracy of routine laboratory diagnosis of malaria in the United Kingdom. Journal of Clinical Pathology, 47(8), 740–742.
10. World Health Organization. (2000). New perspectives in malaria diagnosis (WHO/MAL/2000.1091). World Health Organization.
11. Ovuakporaye, S. I., Enaohwo, M. T., Mordi, J. C., & Naiho, A. O. (2020). Serum electrolytes and renal histology of Wistar rats treated with seed extract of Citrullus lanatus. Journal of Pharmacy and Bioresources, 17(1), 66–74. https://doi.org/10.4314/jpb.v17i1.11
12. John, S. M., Sundarsanam, A., Sitaram, U., & Moody, A. H. (1998). Evaluation of OptiMAL, a dipstick test for the diagnosis of malaria. Annals of Tropical Medicine and Parasitology, 92(6), 621–622.

13. Moody, A., Hunt-Cooke, A., Gabbett, E., & Chiodini, P. (2000). Performance of the OptiMAL malaria antigen capture dipstick for malaria diagnosis and treatment monitoring at the Hospital for Tropical Diseases, London. British Journal of Haematology, 109(4), 891–894.
14. Oduola, A. M., Omitowoju, G. O., Sowunmi, A., Makler, M. T., Falade, C. O., Kyle, D. E., Fehintola, F. A., Ogundahunsi, O. A., Piper, R. C., Schuster, B. G., & Milhous, W. K. (1997). Plasmodium falciparum: Evaluation of lactate dehydrogenase in monitoring therapeutic response to standard antimalarial drugs in Nigeria. Experimental Parasitology, 87(3), 283–289.
15. Albertini, A., Lee, E., Coulibaly, S. O., Sleshi, M., Faye, B., Mationg, M. L., Ouedraogo, K., Tsadik, A. G., Feleke, S. M., Diallo, I., & Talisuna, A. (2012). Malaria rapid diagnostic test transport and storage conditions in Burkina Faso, Senegal, Ethiopia, and the Philippines. Malaria Journal, 11, Article 406. https://doi.org/10.1186/1475-2875-11-406
16. Zimmerman, P. A., & Howes, R. E. (2015). Malaria diagnosis for malaria elimination. Current Opinion in Infectious Diseases, 28(5), 446–454.
17. Plucinski, M., Dimbu, R., Candrinho, B., Rogier, E., Mumbengegwi, D., Kinney, R., Nyatanyi, T., Kachur, S. P., & Halsey, E. S. (2017). Malaria surveys using rapid diagnostic tests and validation of results using post hoc quantification of Plasmodium falciparum histidine-rich protein 2. Malaria Journal, 16, Article 451.
18. Doritchamou, J. Y. A., Akuffo, R. A., Moussiliou, A., de Tove, Y. S. S., Houngbegnon, P., Ahouangninou, C., & Massougbodji, A. (2018). Submicroscopic placental infection by non-falciparum Plasmodium spp. PLoS Neglected Tropical Diseases, 12(3), e0006279. https://doi.org/10.1371/journal.pntd.0006279
19. World Health Organization. (2015). How malaria rapid diagnostic tests work. World Health Organization.
20. Dalrymple, U., Arambepola, R., Gething, P. W., & Cameron, E. (2018). How long do rapid diagnostic tests remain positive after antimalarial treatment? Malaria Journal, 17, Article 228. https://doi.org/10.1186/s12936-018-2382-3
21. World Health Organization. (2018). Malaria rapid diagnostic test performance: Results of WHO product testing of malaria RDTs: Round 8 (2016–2018). World Health Organization.
22. Cheng, Q., Gatton, M. L., & Barnwell, J. W. (2014). Plasmodium falciparum parasites lacking histidine-rich protein 2 and 3: A review and recommendations for accurate reporting. Malaria Journal, 13, Article 283.
23. Gamboa, D., Ho, M. F., Bendezu, J., Torres, K., Chiodini, P. L., Barnwell, J. W., & Cheng, Q. (2010). A large proportion of Plasmodium falciparum isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: Implications for malaria rapid diagnostic tests. PLoS ONE, 5(1), e8091. https://doi.org/10.1371/journal.pone.0008091
24. Watson, O. J., Slater, H. C., Verity, R., Parr, J. B., Mwandagalirwa, M. K., Tshefu, A., & Ghani, A. C. (2017). Modelling the drivers of the spread of Plasmodium falciparum hrp2 gene deletions in sub-Saharan Africa. eLife, 6, e25008. https://doi.org/10.7554/eLife.25008
25. Gendrot, M., Fawaz, R., Dormoi, J., Madamet, M., Pradines, B., & Bogreau, H. (2019). Genetic diversity and deletion of Plasmodium falciparum histidine-rich protein 2 and 3: A threat to diagnosis of P. falciparum malaria. Clinical Microbiology and Infection, 25(5), 580–585.
26. World Health Organization. (2017). Malaria threat maps. World Health Organization.