Research Article
Volume 11:4, 2022

Journal of Malaria Control and Elimination

ISSN: 2470-6965 Open Access

Is the Current Diagnostic Performance of Three Selected Malaria Rapid Diagnostic Kits in Bono Region in Ghana Comparable to Malaria Microscopy, the Gold Standard?

Ofosu Ntiamoah David[1,2*], Asamoah Akwasi[1], Tuekpe Mawuli Ransford[1], Ankoma Rachel[1], Amaniampong Nana Boahemaa[1] and Yeboah Freda[1]

[1]Department of Basic and Applied Biology, School of Sciences, University of Energy and Natural Resources, Sunyani, Ghana

[2]Department of Medical Diagnostics, Faculty of Allied Health Science, Kwame Nkrumah University of Science and Technology, Ghana

Abstract

Background: Malaria is considered one of the highest killer diseases caused by a protozoan parasite of a severity of genus Plasmodium. The symptoms of malaria appear similarly with symptoms of other diseases and have posed a huge problem in terms of accurate and rapt diagnosis. The purpose of this study was to compare the sensitivity and specificity values of two diagnostic tools; malaria microscopy and rapid diagnostic test kits (using three different RDTs) in the diagnosis of malaria.

Methods: A cross-sectional study was performed on patients at the Holy Family Hospital at Berekum in the Bono East Region of Ghana. One hundred and fifty-five (155) participants were recruited. Their blood samples were taken. The participants were tested for malaria using the Histidine Rich Protein-2 (HRP-2) antigen based RDTs (Carestart, SD-Bioline and First Response) and malaria microscopy was also performed for all participants.

Results: All three RDTs had a 100% sensitivity value and a 98.5% specificity value. The reported positive predictive value and negative predictive values were 92.5% and 100% respectively for all RDTs. All three RDTs had a 95.4% agreement with microscopy.

Conclusion: The studies showed that malaria RDTs used in the region and microscopies were comparable in the diagnosis of malaria.

Keywords: mRDT • Microscopy • Ghana • Malaria

Introduction

Malaria is an infectious disease caused by plasmodium parasites. The parasites are spread to people through the bites of the female anopheles mosquito. There are five species that cause malaria in humans namely; *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium vivax* and *Plasmodium knowlesi* and two of those species - *P. falciparum* and *P. vivax* pose the greatest threat. Malaria is amongst the leading causes of death and illness worldwide especially within the tropics and sub-tropics. It's long-term disease which has evaded eradication and continues to cause diseased condition, resulting in death mostly in young children, immune compromised individuals, the aged, poverty-stricken population and pregnant women.

The eradication of malaria especially in endemic area has posed problems in terms of diagnosis; accurate and prompt diagnosis, technical manpower and availability of reagents for test procedure [1]. Diagnosis of the disease is harder in endemic areas because of financial challenges and transmission of infection is quick because of poor living conditions. Malaria poses itself with different symptoms starting from fever to chills, headache, excessive sweating, pain, shivering. These symptoms are common to many disease conditions in their acute phase therefore the recommendation from WHO is that treatment of malaria should be done after proper laboratory diagnosis.

The World Health Organization recommends that malaria treatment be based on demonstration of the infecting *Plasmodium* parasite species. Diagnosis of plasmodium species is usually done via peripheral blood film examination under a microscope. This is the 'Gold standard' of malaria diagnosis [2]. Accurate diagnosis of malaria is important to reduce the morbidity and mortality associated with the condition, while avoiding unnecessary use of antimalarial agents.

Malaria Rapid diagnostic test kits were developed to aid in the quick diagnosis of malaria especially in resource limited settings [3]. These RDTs are based on the detection of specific antigens produced by malaria parasites. These rapid diagnostic test kits are mostly utilized in endemic areas as alternative methods for microscopy. These test kits can be used for detecting all species of malaria parasites. As malaria is one of the major health threats to humans and requires urgent need for rapid diagnosis and treatment for its eradication. However, there are such a large number of brands of RDT test kits on the market, where most of them provide a false negative or false positive result especially for patients with low parasitemia [4]. The resulting inaccuracy in diagnosis increases the morbidity of the disease in the populace and consequently its mortality. There is therefore the need to determine limits and strengths in terms of specificity and sensitivity of the RDT's on the market in order to ensure proper diagnosis and treatment of the disease. This study therefore sought to determine the sensitivity and specificity of three commonly used malaria RDT's in Sunyani Municipality as compared to the gold standard of malaria diagnosis (microscopy).

***Address for Correspondence:** Ofosu Ntiamoah David, Department of Basic and Applied Biology, School of Sciences, University of Energy and Natural Resources, Sunyani, Ghana; E-mail: ntiamoah13@gmail.com*

Date of Submission: 04 July, 2022, Manuscript No. MCCE-22-68448; **Editor Assigned:** 07 July, 2022, Pre-QC No. P-68448; **Reviewed:** 14 July, 2022, QC No. Q-68448; **Revised:** 21 July, 2022 Manuscript No. R-68448; **Published:** 28 July, 2022, DOI: 10.37421/2470-6965.2022.11.186

Materials and Methods

Study design

A cross-sectional study design was used. Participants were recruited by written informed consent.

Study site

The study was conducted on the outpatients and in-patients at the Ward of the Holy Family Hospital in the Bono region, Ghana.

Recruitment of participants

Patients presenting to the out-patient department were recruited via written informed consent to participate in the study.

Inclusion criteria

Patients who were 18 years and above were selected to participate.

Blood sample collection

Whole blood samples (2 ml) were taken from participants into EDTA tubes through a venipuncture sampling method using a 5 cubic centimeter (5 cc) needle and syringe.

Laboratory procedures

Microscopy: Thick and thin films blood films were prepared on the same slide. The thin smear was fixed in absolute alcohol and both smears were allowed to air dry completely. The smears were then stained with a 3% Giemsa working solution for 20 minutes. The slides were screened for *P. falciparum* parasites by two independent technicians (double-blind). Discordance in the diagnostic (positivity/negativity) was solved by a third reader. For the positive slides, the trophozoite stages identified were quantified against 200 white blood cells, using the relative white blood cell count of 8,000/µl as recommended by WHO.

Rapid diagnostic test (immunochromatographic method)

The three test kits used for our study were; Carestart (Access Bio, Ethiopia), SD- Bioline (Standard Diagnostics, Inc. Giheung-gu, Republic of Korea) and First response Premier Medical Corporation Nani Daman and Sarigam, India.

The first response malaria kit used detected the presence of *P. falciparum* specific histidine rich protein-2 (HRP-2). The Carestart kit also detected pan-specific antigen lactate dehydrogenase of *P. falciparum, P. vivax, P. malariae* and *P. ovale*. The SD-Bioline kit detected HRP-2 and *P. falciparum* specific Pldh.

Procedure

Whole blood samples were collected from 155 patients that visited the out-patient department. Each sample was tested using the three (3) kits. To perform the malaria test, 5 µl of whole blood was collected with the provided capillary pipette and transferred to the sample well. Four drops of the assay diluent were added to the diluent well according to the manufacturer's protocol. The results were read after 15 minutes.

Data analysis

Results obtained were entered into Microsoft Excel and Word document. Research Data was analyzed using the Statistical Software SPSS version 25 and results presented in tables. A p-value <0.05 was considered statistically significant.

Table 1. Results of the different methods for the detection of Malaria parasites.

Method	Total (n)	Negative [n (%)]	Positive [n (%)]
Microscopy	155 (100%)	130 (83.9)	25 (16.1)
Care start	155 (100%)	128 (82.6)	27 (17.4)
SD Bioline	155 (100%)	128 (82.6)	27 (17.4)
First Response	155 (100%)	128 (82.6)	27 (17.4)

Results

A total of 155 participants were diagnosed for malaria using microcopy (standard method) and the results were compared to three RDTs (care start, SD Bioline and First Response). Of the 155 participants diagnosed by microscopy, 83.9% tested negative for malaria whilst the remaining participants (16.1%) tested positive for malaria (Table 1). When participants were diagnosed with Care Start RDT kits, 82.6% of the total participants tested negative and the remaining participants tested positive (17.4%) for malaria. Similar trend (82.6% negative *vs*. 17.4% positive) was observed for SD Bioline and First response malaria diagnostic Kits. Table 1 displays the results of the different methods for the detection of malaria parasites.

Malaria microscopy and speciation

Of the 155 participants that were diagnosed by microscopy, no malaria parasites were seen among majority of the participants (83.2%), *P. falciparum* was diagnosed among 11.6% of the participants whilst *P. malariae* was diagnosed among 5.2% of the participants (Figure 1).

Result comparison between the three RDT methods and microscopy for the detection of malaria parasites

The results of the three RDTs were compared to microscopy, which is the standard method of diagnosing malaria. One hundred and thirty (130) participants tested negative for malaria whilst 25 tested positive for malaria. Of the 130 participants that tested negative for malaria, the Care Start RDT kit diagnosed 128 (95%) as negative whilst the remaining 2 participants (1.5%) were falsely classified as positive. However, all the 25 participants (100.0%) that were diagnosed as malaria positive by light microscopy were all also diagnosed as positive by the Care Start RDT kit. Similar trend was observed when SD Bioline and First Response RDT kits were used. The results comparison between the three RDT kits and Microscopy is displayed in Figure 2 (Table 2).

Diagnostic performance of three malaria RDTs compared to microscopy in the detection of malaria parasites

When the performance of Care Start, SD Bioline and First Response RDT kits were compared to microscopy, all the three kits had the same performance. In this study, Care Start, SD Bioline and First Response RDT kits were 100% sensitive, 98.5% specific and with 92.5% positive predictive value and 100% negative predictive value (Table 3). Moreover, all the three RDT kits had significant kappa statistics ($p < 0.0001$) (Table 3).

Diagnostic agreement between the three RDT kits and microscopy

When Cohen's Kappa was run for the inter-rater agreement between the different RDT kits and microscopy, Care Start, SD Bioline and First Response RDT kits were in agreement with 95.4% of microscopy results (Figure 3).

Discussion

Accurate *Plasmodium* species identification is critical not just for determining the appropriate treatment regimen, but also for implementing successful malaria control measures in endemic areas. Misidentification of *Plasmodium* species might cause serious public health problems owing to ineffective treatments, resulting in recrudescence and possibly medication resistance [5]. Malaria control necessitates the use of a high-quality diagnostic technique to detect the parasite before administering anti-malarial therapy in accordance with WHO guidelines [6]. Malaria parasitological diagnosis helps to focus therapy, characterize treatment response and identify the parasite early.

Malaria is one of the most serious public health issues in Ghana and it remains a significant source of morbidity, with majority of the population afflicted with malaria parasites. *Plasmodium falciparum* is responsible for 90%

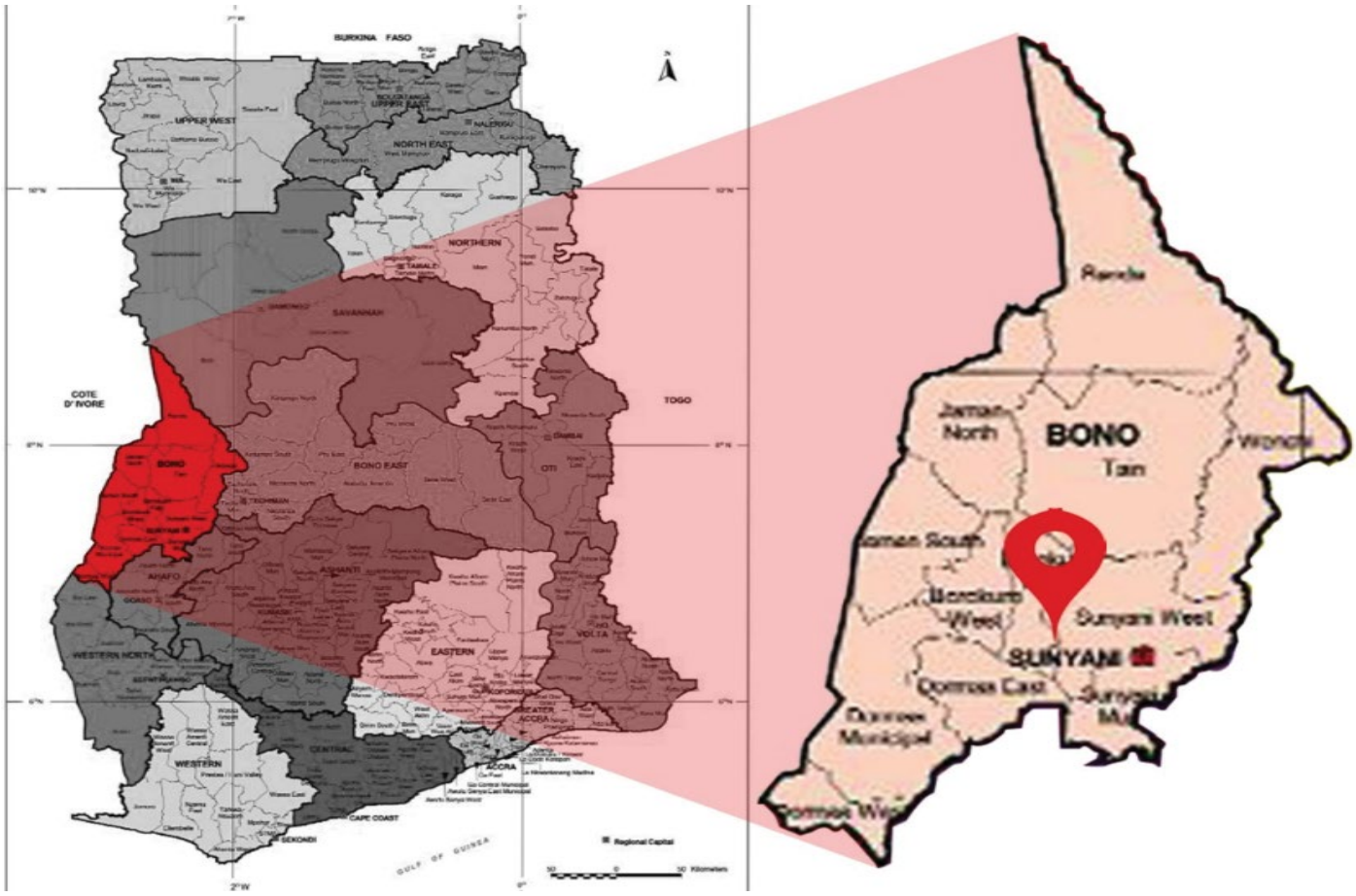

Figure 1. Map showing study site Source: ("Know the 16 regions of Ghana" 2021).

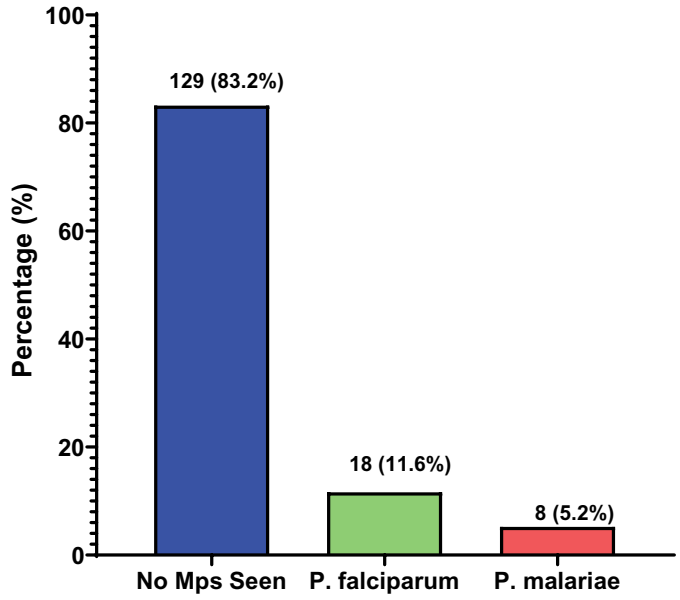

Figure 2. Distribution of microscopic diagnosis and speciation of malaria parasites (P – *Plasmodium* Mps- Malaria parasites).

Table 2. Results comparison between the different RDT methods and Microscopy for the detection of Malaria parasites.

Other Methods of Diagnosing Malaria		Microscopy (Standard Method)	
		Negative (n =130)	Positive (n = 25)
Care Start	Negative	128 (98.5)	0 (0.0)
	Positive	2 (1.5)	25 (100.0)
SD Bioline	Negative	128 (98.5)	0 (0.0)
	Positive	2 (1.5)	25 (100.0)
First Response	Negative	128 (98.5)	0 (0.0)
	Positive	2 (1.5)	25 (100.0)

Table 3. Diagnostic performance of three malaria RDTs compared to microscopy in the detection of malaria parasites.

Method	Specificity (95% CI)	Sensitivity (95% CI)	PPV %	NPV %	κ	*p-value*
Care Start	98.5 (94.5-99.7)	100.0 (86.6-100.0)	92.5	100	95.4	< 0.0001
SD Bioline	98.5 (94.5-99.7)	100.0 (86.6-100.0)	92.5	100	95.4	< 0.0001
First Response	98.5 (94.5-99.7)	100.0 (86.6-100.0)	92.5	100	95.4	< 0.0001

CI: Confidence Interval; PPV-Positive Predictive Value; NPV: Negative Predictive Value; κ: Kappa, p-values reported the significance of statistics.

of the malaria load. This study investigated the test performance of two routine malaria diagnostic methods (microscopy technique and Rapid Diagnostic Tests (RDTs). However, three different RDT kits were used in this study with microscopy as the gold standard.

In this study, mRDTs was able to detect 2 samples microscopically confirmed negative. Samples negative by microscopy but positive by mRDTs could represent samples collected from participants already on antimalarial drugs. Such samples will not have intact parasites but the gene product of *hrp2* gene will still be in circulation [7]. Such samples will test positive by mRDTs but microscopically they will show negative. Some studies have shown that HRP2 antigens could still remain in circulation for as long as 31 days following treatment [8].

The sensitivity and specificity of the apparatuses were also used to assess

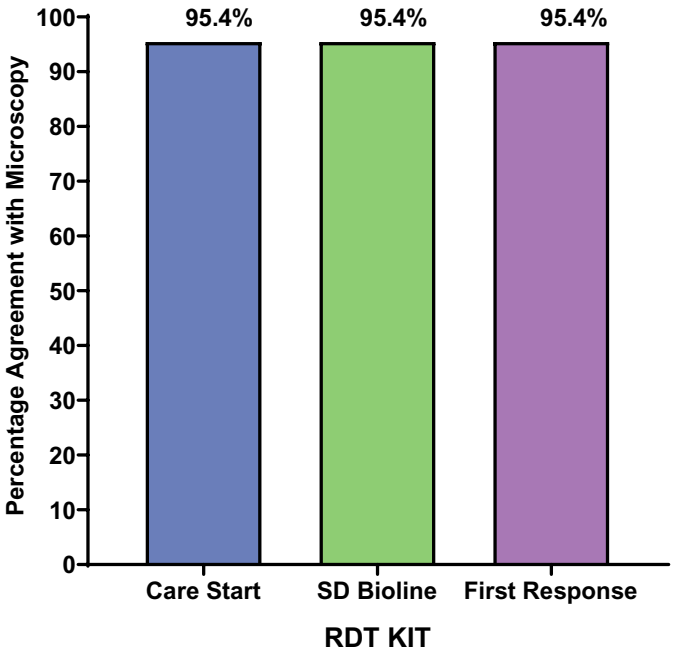

Figure 3. Percentage agreement between the three RDT kits and microscopy.

the RDTs' discriminatory accuracy against microscopy. The overall sensitivity and specificity among the three diagnostic mRDTs were equal. The sensitivity and specificity were respectively 98.5% and 100%. This study found a higher rate of RDT sensitivity, implying a low proportion of false negative results. Despite light microscopy's inherent limitations [9], the diagnostic sensitivity was still excellent when compared to the mRDTs. The sensitivity of the malaria RDT was within the WHO's recommended sensitivity level of ≥ 95%. When compared to the 88.6% sensitivity reported by Mahende C, et al.[10] in Tanzania and Ojurongbe O, et al. [11] in Nigeria, the RDT had a higher sensitivity. Numerous variables, including storage humidity, temperature, product quality and end-user operation, might impact the accuracy of RDTs diagnostic. In any location where this method is utilized, good sensitivity and a negative RDT result would allow malaria to be ruled out, eliminating needless presumed therapy. This study's 98.5% specificity matches that of in Ibadan, Nigeria, who found 99.6% specificity. In a research conducted in Port Harcourt, Nigeria Murungi, et al. [12] observed lower specificities of 56.26% and 46.7%, respectively, in Nigeria and western Uganda.

The mRDTs had a positive predictive value of 92.5% and a negative predictive value of 100%. The 92.5% positive predictive value achieved in this investigation matches the findings of Albertini A, et al [13]. Implying that 9 out of 10 who tested positive with this RDT also tested positive for malaria using the gold standard. The incidence of malaria illness in the examined community, however, has an impact on positive and negative predictive values.

Despite the fact that microscopy is considered the gold standard, there is still the possibility of human error, which might account for the discrepancy. Microscopy however enables for the estimation of parasite densities and the identification of all species while being less expensive than other approaches [14]. As a result of the high specificity, the cost of malaria treatment will become more inexpensive, increasing the number of non-infected people who would otherwise go undiagnosed, necessitating the requirement for adequate case definition and management.

Recommendation

Per the data from this study, it is recommended that RDTs should be used in conjunction with malaria microscopy and in rural areas where the lack expertise and equipment, RDTs only can be used in the diagnosis of malaria. Physicians should also enquire from patients if they are taking or have taken any anti-malarial drug before requesting for malaria RDTs since it can affect the result. All positive cases can further be tested using the polymerase chain reaction method (PCR).

It is recommended that more research be carried out into finding less time consuming yet highly specific and sensitive in the diagnosis of malaria.

Conclusion

In conclusion, malaria rapid diagnostic tests are rapid, simple and very convenient for the diagnosis of malaria. Many other effective and acceptable diagnostic tools are being studied so as to grasp the best diagnostic tool for the diagnosis of malaria. This study has shown that malaria RDTs have a higher sensitivity and specificity value and provides results faster than malaria microscopy and also don't require expertise to perform the test.

Due to the advantages of the rapid diagnostic tests, they should be made available in endemic areas especially the remote areas where microscopy is difficult to help in early detection of the infection. However, microscopy still remains the gold standard and should be referred to as much as possible. The study has also shown that although a small percentage of the RDTs gave a false positive result, it wasn't significant as most of the patients that tested negative and positive were true negative and true positive when microscopy was performed.

What is Already Known on this Topic?

Some studies have reported high sensitivity of mRDT used in the big cities in Ghana.

What this Study Adds

The mRDT kits available for use in the three BONO regions of Ghana have high sensitivity and specificity.

Competing Interests

The authors declare no competing interest.

Authors' Contributions

DNO, RMT, AR, ANB YF, AA conceived and designed the study, also collected and transferred all data from the field, did laboratory work and drafted the manuscript AR, ANB, YF undertook the experimental testing in the laboratory. DNO and AA reviewed the study design, methodology and critically reviewed the manuscript.

Acknowledgement

We wish to acknowledge the entire staff of Department of Basic and Applied Biology for their support during this work. Special thanks go to the laboratory technicians whose guidance led to the success of this work.

Ethical Approval and Participant Consent

Ethical approval was obtained from the Committee for Human Research and Ethics (CHRE) of the University of Energy and Natural Resources (CHRE/CA/015/033). The aims, benefits, risks and right of withdrawal at any time from the study was well explained to the study participants in English and in the Local Dialect (mostly Twi) and their consent was obtained.

Consent for Publication

All authors have duly consented for this manuscript to be submitted for publication

Availability of Data and Materials

The data for this study is available and will be provided upon request.

Funding

This study was wholly funded by the authors of the manuscript. There was no external funding.

References

1. Cui, Liwang, Guiyun Yan, Jetsumon Sattabongkot and Bin Chen, et al. "Challenges and prospects for malaria elimination in the Greater Mekong Subregion." *Acta Trop* 121 (2012): 240-245.
2. Bharti, Ajay R., Kailash P. Patra, Raul Chuquiyauri and Margaret Kosek, et al. "Polymerase chain reaction detection of Plasmodium vivax and Plasmodium falciparum DNA from stored serum samples: Implications for retrospective diagnosis of malaria." *Am J Trop Med Hyg* 77 (2007): 444-446.
3. Hansen, Kristian S., Eleanor Grieve, Amy Mikhail and Ismail Mayan, et al. "Cost-effectiveness of malaria diagnosis using rapid diagnostic tests compared to microscopy or clinical symptoms alone in Afghanistan." *Malar J* (2015): 1-15.
4. Tahar, Rachida, Collins Sayang, Vincent Ngane Foumane and Georges Soula, et al. "Field evaluation of rapid diagnostic tests for malaria in Yaounde, Cameroon." *Acta Trop* 125 (2013): 214-219.
5. Jun-Mo, Kang., Cho, P.Y., Moe, M. and Lee, J., et al. (2017). Comparison of the diagnostic performance of microscopic examination with nested polymerase chain reaction for optimum malaria diagnosis in Upper Myanmar. *Malar J 16*(1), 1-8.
6. Falade, Catherine O., IkeOluwapo O. Ajayi, Jesca Nsungwa-Sabiiti and Mohamadou Siribié, et al. "Malaria rapid diagnostic tests and malaria microscopy for guiding malaria treatment of uncomplicated fevers in Nigeria and prereferral cases in 3 African countries." *Clin Infect Dis* 63 (2016): S290-S297.
7. Baker, Joanne, James McCarthy, Michelle Gatton and Dennis E. Kyle, et al. "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 (2005): 870-877.
8. Kain, Kevin C., Dennis E. Kyle, Chansuda Wongsrichanalai and Arthur E. Brown, et al. "Qualitative and semiquantitative polymerase chain reaction to predict Plasmodium falciparum treatment failure." *J Infect Dis* 17 (1994): 1626-1630.
9. Mens, P., N. Spieker, S. Omar, M. Heijnen and H.D.F.H. Schallig, et al. "Is molecular biology the best alternative for diagnosis of malaria to microscopy? A comparison between microscopy, antigen detection and molecular tests in rural Kenya and urban Tanzania." *TM & IH* 12 (2007): 238-244.
10. Mahende, Coline, Billy Ngasala, John Lusingu and Tai-Soon Yong, et al. "Performance of rapid diagnostic test, blood-film microscopy and PCR for the diagnosis of malaria infection among febrile children from Korogwe District, Tanzania." *Malar J* 15 (2016): 1-7.
11. Ojurongbe, Olusola, Olunike Olayeni Adegbosin, Sunday Samuel Taiwo and Oyebode Armstrong Terry Alli, et al. "Assessment of clinical diagnosis, microscopy, rapid diagnostic tests and polymerase chain reaction in the diagnosis of Plasmodium falciparum in Nigeria." *Malar Res Treat* 2013 (2013).
12. Murungi, Moses, Travis Fulton, Raquel Reyes and Michael Matte, et al. "Improving the specificity of Plasmodium falciparum malaria diagnosis in high-transmission settings with a two-step rapid diagnostic test and microscopy algorithm." *J Clin Microbiol* 55 (2017): 1540-1549.
13. Albertini, Audrey, Evan Lee, Sheick Oumar Coulibaly and Markos Sleshi, et al. "Malaria rapid diagnostic test transport and storage conditions in Burkina Faso, Senegal, Ethiopia and the Philippines." *Malar J* 11 (2012): 1-9.
14. Berzosa, Pedro, Aida de Lucio, María Romay-Barja and Zaida Herrador, et al. "Comparison of three diagnostic methods (microscopy, RDT and PCR) for the detection of malaria parasites in representative samples from Equatorial Guinea." *Malar J* 17 (2018): 1-12.

How to cite this article: David, Ofosu Ntiamoah, Asamoah Akwasi, Tuekpe Mawuli Ransford and Ankoma Rachel, et al. "Is the Current Diagnostic Performance of Three Selected Malaria Rapid Diagnostic Kits in Bono Region in Ghana Comparable to Malaria Microscopy, the Gold Standard?." Malar Contr Elimination 11 (2022): 186.