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Research Article

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Clinical Utility of Mono-Biomarker based Malaria Rapid Diagnostic Test Kits at a Military Medical Centre in Ghana: A Prospective Pilot Study

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Abstract

Introduction: *The proliferation of non-falciparum species of plasmodium into a predominant falciparum population compromises the utility of monobiomarker-based Malaria Rapid Diagnostic Test (mRDT). This study evaluated the clinical utility of a monobiomarker-based Carestart and Paracheck mRDTs, which were in routine use at a Military Medical Centre in Ghana at the time of the study.*

Methods: *The study was designed to assess the validity of candidate mRDTs among population risk of exposure to non-falciparum species of plasmodium in Ghana. Blood samples collected from a consecutive series of 207 febrile patients in the months of June and July 2020, were tested for malaria parasites, using the mRDTs and microscopy as the gold standard. Prevalence, validity, and reliability metrics were determined using Frequentist, Receiver Operating Characteristics (ROC), and Kappa statistics, respectively.*

Results: *The prevalence was 23.2% and 12.3% using microscopy and candidate mRDT, respectively. Sensitivities and specificities were 53.2% and 98.1% (Carestart) 45.8% and 99.4% (Paracheck), respectively. Neither ROC analysis showed a significant disparity between mRDTs (Carestart: AUROC=0.75 vs Paracheck: AUROC=0.73), nor the reliability index showed disagreement between both mRDTs (Cronbach's α = 0.92). However, there was significant disagreement between microscopy and mRDTs (Carestart: Kappa=0.58 vs Paracheck: Kappa=0.55).*

Conclusion: *The use of a monobiomaker mRDTs in this study led to a significant variation between the 'internal' and 'ecological' validity metrics. Averagely, 84% of mRDT false negatives were confirmed by microscopy as non-falciparum species of plasmodium. The observed trends have and research policy implications. It is therefore, critical to accelerate the implementation of WHO's recommendation to switch from mono to multiple biomarker (s) based mRDTs for detecting both falciparum and non-falciparum species. Extended research is needed to consolidate our understanding on the dynamics of malaria among our military personnel exposed to non-falciparum plasmodium.*

Keywords: Diagnostic Malariology, Microscopy, mRDTs, Carestart, Paracheck Internal Validity, Ecological Validity

1. Introduction

The Sustainable Development Goal (SDG 3.3) projected to reduce malaria by 90% in 2030 remains the international focus and determination to eradicate malaria [1]. Being a subscriber to SDGs conventions, Ghana implemented a wide range of interventional programmes in collaboration with sustainable development partners towards the SDG3.3 milestones and deadlines [2]. These interventions have had a significant impact in reducing malaria morbidity and mortality rates in Ghana before the implementation of 'public health sector strategies" on malaria in 2016 [3]. Specifically, between 2010 and 2020, reductive trends in malaria morbidity of about 13.47 million cases in 2017 to 5.9 million in 2020 were observed in Ghana [4]. Despite this progress and "with less than a decade of actions" to meet the global deadline, Ghana remained classified as a malaria endemic country [3,4]. The factors, which might hinder the expected progress of Ghana towards the 2030 deadline, could be due to inter variations in regional ecologies and transmission patterns in Ghana, in one hand, and in another hand, the use of invalidated source data on malaria, which was originated at the primary health care centers and rooted through the District Health Information Management System (DHIMS2) platform for policy formulation [5-7]. Of interest in this study is the implementation and implications of using malaria rapid diagnostics test (mRDT) kits results to inform policy on malaria management in Ghana. As part of the global health strategy on confirmatory malariology, the use of mRDT kits adopted in 2010, and its operation fully implemented in Ghana by 2014, to mitigate the constraints associated with microscopy at the point of care level [8]. Most of the mRDTs in circulation, were engineered to detect only Plasmodium Falciparum infection, using the antigen "Histidine-Rich Protein 2 (*pfhrp2* / 3)" as a biomarker. To detect the presence of other species of plasmodium infections, a combination of biomarkers, including the antigen "Plasmodium Lactate Dehydrogenase (pLDH)" and "Plasmodium Aldolase (pAldo)" antigen, are used [9]. Although the availability of combined biomarkers-based mRDTs is traceable in sub-Saharan African countries, their use was largely limited to Research institutions and/or private medical centers. However, most of the kits in circulation in the public Health services facilities in sub-Saharan Africa, including Ghana were monobiomaker (*pfhrp2*/3)-based mRDTs, which is engineered to detect only Plasmodium Falciparum infection. However, due to growing evidence on changing epidemiology of the heterogeneity of *pfhrp2* and *pfhrp3* biomarkers on one hand, and the emergence of non-falciparum species of plasmodium infections in naïve population on another hand, WHO recommends the use of mRDT based on non-*pfrp2* biomarker, if the detection threshold of *pfhrp2 / 3 delated* parasites is below 90% confidence interval of 90% prevalence; or complete switch to non-*pfrp2*-based mRDTs if the prevalence of muted parasite is >5% [10,11]. To contribute to the baseline data needed to support the implementation and application of this recommendation in Ghana, we evaluated the clinical utility of Care Start and Paracheck mRDTs, which are mono biomarkers, in routine use among a strategic population that might have been exposed to non-falciparum species of plasmodium infections in the Ho Municipality.

2. Materials and Methods

2.1. Study Design

This was as an exploratory pilot survey, designed to test the field "ecological validity" vis-a-avi the manufacturers' "Internal Validity" of the named mRDTs as an equivalence and/or non-inferior to microscopy at the primary health care level in Ghana.

2.2. Study Settings

The study was carried out at the 7 Medical Reception Station, which is a military hospital located in Ho, on coordinates 6.617494 and 0.4875669 [Figure 1c], [12,13]. The Ho Municipality is an area of about 573.2 km² (Figure 1b) [12]. The population is estimated to be 180,420; population density of 314.8/ km2 and annual population Change was 0.6% [12]. The two main rainy seasons include a 'major rain' seasons (April-July) and a 'minor rain' seasons (September–November) [12]. The mean monthly temperature in the Municipality ranges from 22.0°C to 32.0°C (71.6°F and 89.6 °F). The Municipality experiences an unremitting malaria transmission with some seasonal peaks coinciding with the rainy seasons, with the highest malaria cases usually observed one month after the start of the rainy season [14]. The facility was established in 1967 as a sick bay for military staff and families stationed at the 66 Artillery Regiment. In 2008, the facility was upgraded to a primary health care Centre and opened to the general public in the Ho Municipality.

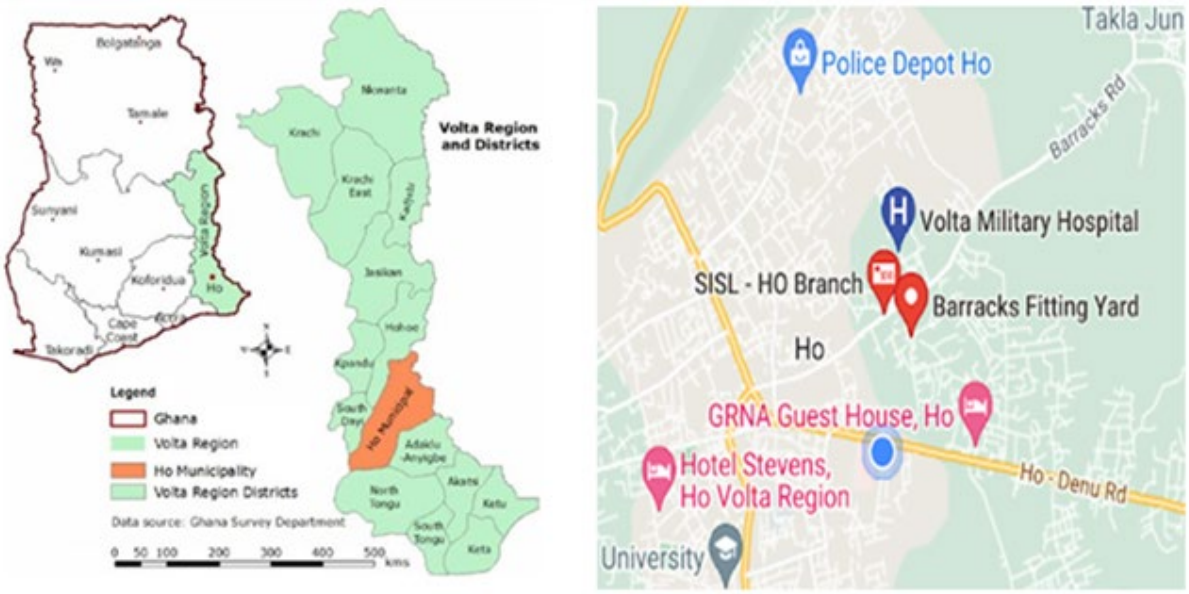

Figure 1: The Study Setting Showing Maps of Ghana (1a), Ho Municipality in The Volta Region, And Google Location of Military Hospital (7 Medical Reception Centre) in Ho Municipality where the Study was Conducted

2.3. Study Participants

An average of 444 malaria patients estimated to attend the clinic within a two-month duration constituted the study population. Using Raosoft software that was scaled at 5% margin of error, 95% confidence interval, and 50% response distribution 207 were determined as the minimum sample size. The sampling was a consecutive series of patients clinically diagnosed with malaria within the study period (June -July 2020).

2.4. Data Collection, Management and Analysis

2.4.1. Laboratory Procedures

The candidate mRDTs used were Carestart® (Access Bio, Inc. NJ, USA) and Paracheck® (Orchid Biomedical Systems, Goa, India). Both RDTs are Plasmodium Falciparum Histidine Rich Protein-2 (*pfhrp-2*) based Immuno Chromato Graphic RDTs. The 'Internal Validity' values as captured on manufactures' leaf-let were 98% sensitive and 97.5% specific for Carestart, and 99.4% sensitive and 100% specific for Paracheck. About 3 ml of whole blood sample was taken aseptically from each participant into Dipotassium Ethylene Diamine Tetraacetic Acid (K2EDTA) tube of whole blood from each participant, and malaria infection was tested according to the manufactures' instructions for each candidate mRDTs. The thick and thin blood film on the same glass slides of the same blood was tested for malaria parasites by microcopy according to WHO protocol [15]. To overcome biases due to observational errors, three microscopists were used. Two of the microscopists were stationed at the study site and an expert microscopist (WHO accredited) was stationed at the Department of Medical Laboratory Sciences at the University of Health and Allied Sciences.

2.4.2. Data Management and Statistics

The data was managed electronically using the Microsoft Visual basic platform. Data entry was quality controlled using the double data entry mechanism. The clean data were exported to Stata version 13.0 (Stata Corp. College Station, TX, USA) for statistical analyses. The prevalence was determined using the frequentists test. Validity metrics were determined using the "Java Stat Two-Way Contingency Table" [16]. Positive Predictive Value (PPV) and

Negative Predictive Values (NPV) were determined using "Bayes' theorem". The diagnostic precision of both mRDTs relative to microscopy was evaluated using an 'Operating Characteristic Curve' (ROC) analysis. The reliability metrics were determined using "kappa statistics". The inter-rater reliability and internal consistency of the two RDTs were determined using 'Intraclass correlation coefficient" (ICC) and "Cronbach's alpha" statistics. .

3. Result

The prevalence of malaria was determined as 23.2%, 13.5% and 11.1% using microscopy, CareStart® and Paracheck™ RDTs respectively (Table1,section A). Furthermore, out of 26 cases, determined as negative by Paracheck™, 21 (80.8%) were confirmed by microscopy as non-falciparum species of plasmodium (Table1, section A). Similarly, of 22 cases determined as negative by Carestart®, 19 (83.6%) were confirmed by microscopy as belonging to non-falciparum species of plasmodium (Table1, section B). Relative to microscopy, the true probability (TP) of malaria infection using Paracheck™ and Carestart® kits were 22 and 25 respectively. Inversely, the true negative (TN) of malaria infection using Paracheck™ and CareStart® were 158 and 157 respectively (Table 1, Section D). Therefore, the sensitivities for the Paracheck™ and CareStart® kits were 45.8% (CI 95%: 31, 60.8) and 53.2% (CI95%: 38.1, 67.9), respectively [Table 1, Section D]. The specificity of ParaCheck and CareStart® were 99.4% (CI 95%: 96.5, 100.0) and 98.1% (CI 95%: 94.6, 99.6), respectively. The Positive Predictive Values (PPV) for Para check™ were 95.7 % (CI 95%:75.3, 99.4) and Care Start®: 90.0 % (CI95%: 72.5, 96.3). Negative predictive values (NPV) for Para Check™ and Care Start® were 85.9 % (CI 95%: 82.4, 88.7) and 87.7% (CI 95%: 84.0, 90.6), respectively.

A. Prevalence of Malaria by the methods used for malaria diagnosis		
Test Outcomes	**Microscopy**	**Paracheck™**
	Cases (%)	**Cases (%)**
❖ Malaria Positive:	48 (23.2%)	23 (11.1%)
❖ Malaria Negative:	159 (76.8%)	184 (88.9%)
❖ Overall Total	207 (100%)	207 (100%)
B. Assessment of malaria test outcomes by RDTs and Microscopy as a reference		
• True Positives	-	22
• False Positives	-	1
• True Negatives:	-	158
• False Negative	-	26
C. Frequentists of False Negative mRDT examined by expert microscopy		
➢ P.falciparum:	27 (56.3%)	05 (19.2%)
➢ P. malariae	10 (20.8%)	10 (38.5%)
➢ P. ovale	07 (14.6%)	07(26.9%)
➢ P. vivax	04 (08.3%)	04(15.4%)
➢ Sub-Total	48.0 (100%)	26 (100%)
D.Validation and Predictability tests on candidate mRDTs		
i. Accuracy metrics-	**Paracheck™**	**Carestart®**
✓ % Sensitivity (CI95%)	45.8 (31.4-60.8)	53.2(38.1-67.9)
✓ % Specificity (CI95%)	99.4(96.5-100.0)	98.1(94.6-99.6)
ii. Predictability metrics		
✓ % PPV (CI95%)	95.7(75.3-99.4)	85.9(82.4-88.7)
✓ % NPV (CI95%)	90.0(72.5-96.3)	87.7(84.0-90.6)
iii. Likelihood Ratio Estimation		
✓ PLR (CI95%)	72.9(10.1-526.7)	28.4(9.0-89.8)
✓ NLR (CI95%)	0.5(0.4-0.7)	0.5(0.4-0.6)
Key: P= Plasmodium; RDT= Rapid Diagnostic Test; VMH=Volta Military Hospital. The number of cases are presented as frequencies, and the percentages (%) in parenthesis. CI95% = 95% Confidence Intervals; TP = True positive, FP = False positive, TN = True negative, FN = False negative, PPV = Positive predictive value, NPV = Negative predictive value, PLR = Positive Likelihood Ratio, NLR = Negative likelihood ratio n = Number of patients		

Table 1: Frequentist and Validation Tests on Malaria Diagnostic Methods at V.M.H in 2020

Furthermore, as shown in Table 2, the kappa values of 0.55 (CI 95%: 0.41, 0.69) scored between Para Check™ and microscopy on one hand, and 0.58 (CI 95%: 0.45, 0.73) between Care Start® and microscopy in another hand, affirmed that both RDTs were less reliable compared to microscopy in diagnosis of malaria infection and disease in this study. However, neither there was a significant intra variation in diagnostic accuracy metrics between Para check™ and Care Start®, nor was there a significant intra variation in reliability metrics between Para check™ and Care Start®. The difference between AUROC and both RDTs was 0.03 (CI 95%: -0.02, 0.071; p=0.289) (Figure2), and the Kappa score between both RDTs was 0.84 (CI 95%: 0.84, 0.96) (Table2).

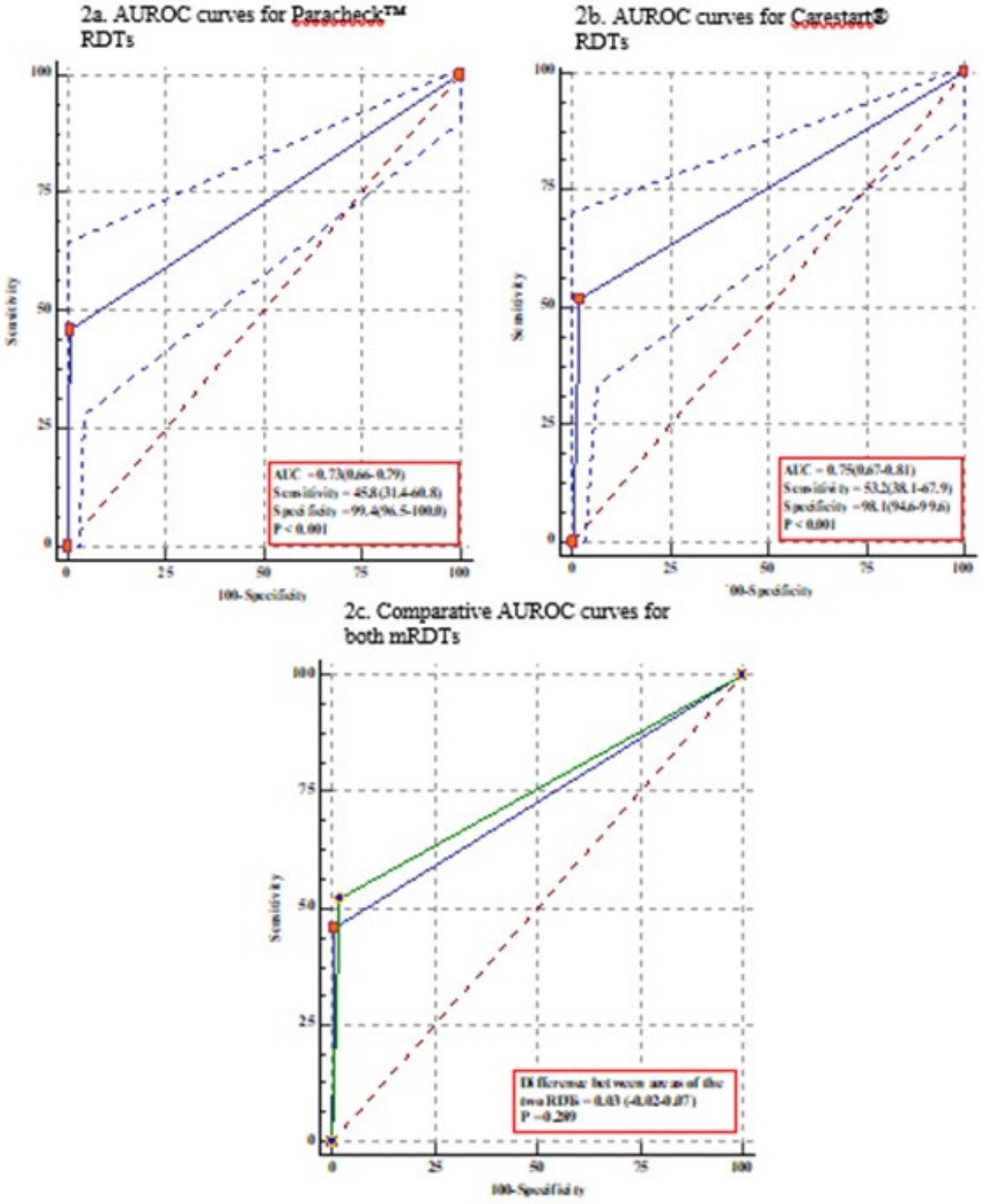

Figure 2: Area Under Receiver Operating Characterisrics (AUROC) Curve Analysis and Ecological Validity for Paracheck™ RDT (a), Care Start® RDT (b), and between both RDTs (c) Compared to the Gold Standant (Microscopy) for Diagnosis of Malaria Infection among Study participants clinically diagnosed for malaria in Volta Military Hospital in 2020.The AUROC) value of <0.7 depicts a scale of unacceptable, 0.7-0.8 (acceptable), 0.8-0.9 (excellent) and >0.9 (outstanding).

Candidate RDT Kits	Microscopy* Kappa scores (CI95%)	RDT Paracheck™ Kappa score (CI95%)
Paracheck™	0.55 (0.41 - 0.69)	
Carestart®	0.58 (0.45 - 0.73)	0.84 0.73 To 0.96)
Weighted Kappa values: < 0.20 = Poor; 0.21 - 0.40 = Fair; 0.41 - 0.60 = Moderate; 0.61 - 0.80 = Good, and 0.81 -1.00 = Very good. * = Gold Standard (reference test method); CI95% = 95% Confidence Interval in parentensis		

Table 2: Inter-Rater and Intro-Rater Reliability Analysis using Cohen's Kappa Statistic

Cronbach's alpha score of 0.9 (CI 95% = 0.90 – 0.94) affirmed that the intrinsic mechanism for consistency in malaria diagnosis was reliably the same between Para checkTM and Care Start® (Table3). As demonstrated in Figure 2, the AUC values of 0.73 (CI 95% = 0.66 – 0.79) scored between Para checkTM and microscopy on one hand, and 0.75 (CI 95% = 0.67 – 0.81) between Care Start and microscopy on another hand depicted a significant inter variation in diagnostic accuracies between both RDTs and microscopy. Therefore, both Para checkTM and Care Start RDTs were significantly non-equivalent and/or inferior to microscopy by 27% ($p<0.001$) and 25% ($p<0.001$) respectively.

Internal Consistency Reliability Test	Cronbach's alpha	95% Confidence Interval
	0.92	0.90 to 0.94
Intraclass Correlation	Intraclass correlation (degree of consistency)	95% Confidence Interval
• Single measures b	0.85	0.80 to 0.88
• Average measures c	0.92	0.89 to 0.94
Key: b= index of reliability from a single RDT test; c=index of reliability from an average of testing two RDTs. RDTs= Rapid Diagnostic Test Kits; VMH=Volta Military Hospital.		
Interpretation of Cronbach's Alpha scale: (Lundqvist et al., 2014). • 0.7 – 0.8 = satisfactory • >0.80 = very good; • 0.65-0.80= good • 0.35-0.65=moderate.		

Table 3: Intra Consistency Analysis between Paracheck™ and Carestart® RDTs in VMH in 2020

4. Discussion

The malaria prevalence of 23% using microscopy in our study was within the range of 9.7% in Ketu South to 31% in Adaklu districts recorded in the study region in 2020. However, this rate was significantly higher by 8.1% point above the rate of 15% recorded in the Ho Municipality in the study township in 2020 [17]. This intra-variation could be due to an anecdotal increase in clinical cases of malaria among cohorts of military personnel returning from international peacekeeping in malaria endemic regions. Inversely, the relatively low rate of 15.1% recorded in the DHIMS database for Ho Municipality could be due to limitations associated with mRDTs data in the Municipality or it is a natural reflection of reductive trends in incidence of malaria observed in Ghana [4,14].

In addition, the malaria prevalence of 23% using microscopy was relatively higher than the mean rate of 12.3% using candidate mRDTs in this study. This phenomenon of recording higher rates of malaria infection by microscopy relative to lower rates using CareStart® mRDTs was reported in a number of comparable studies, including Ghana and other African counties [18-20]. The mean rate of 49.5% sensitivity recorded for candidate mRDTs in this study was significantly below the manufacturers' mean rates of 96.2% and WHO's threshold of ≥ 95% [21]. However, the mean rate of 99% specificity recorded was comparable to the manufacturers' mean specificity of 98.8% and the WHO's threshold of ≥ 90% [15].

This phenomenon of lower sensitivity relative to higher specificity rates for mRDTs was also reported in previous multicenter studies in same region and other regions in Ghana as well as studies in other African countries [20,22-24]. Similar to pfhrp2-based CareStart, a relatively lower mean rates of sensitivity (72.7%) and specificity (62.7%) rates for using pfhrp2-based Paracheck were also previously reported in Ghana and West-African countries as well as other African countries [18,25,26]. The most cited reasons for this phenomenon included, but were not limited to, variations in geo-demographics of the clinical population and impact of experience microscopy [18-20,23].

Moreover, the disconcordance between candidate mRDTs and microscopy is mathematically associated with a poor sensitivity of tested mRDTs on the one hand, and low sensitivity rates associated with false negativity of tested mRDTs on another hand [27]. The most cited reasons for false negativity associated with mRDTs are technical, biological, social, infrastructural, regulatory, and economic barriers [27]. As Miller and Sikes (2015) outlined, the "technical barriers" include "susceptibility to heat and humidity", "inadequate characterization of the affinity agent and antigen", "prozone effect", "subjective interpretation of test lines", "time-dependence of signal development", "insensitivity to low disease loads" and "unsuitability for pathogen quantification" [27].

Regarding the factors of "heat and humidity", the storage conditions were traceable in the study laboratory and the metrics were within the normal ranges. However, records of quality control on maintenance of optimum storage condition during transportation from the national through regional storage facilities to the study facility were not traceable for verification. Although there was no gross observation of inaccurate low intensity test lines due to either overabundant antigens or overabundant antibodies, the association between our false negative samples and prozone phenomenon was experimentally not evaluated. However, due to experience of the Medical laboratory Scientists handling the mRDTs, as well as documentary evidence on the internal quality control mechanism of the sample collection, processing and reading of the mRDT, we could rule out the association between our false negative samples and the factor of the 'subjective interpretation of test-lines', on one hand and "time-dependence of signal development', on another hand.

In addition, biologic factors include lack of biomarkers for a specific pathogen, limited ability of the biomarker to track heterogenic genes emitted by drug resistance or mutation and cross-reactivity in conditions such rheumatoid and Schistosomiasis [27]. Of the 9 characteristics of pathogen identification biomarkers, the absence of diagnostic biomarker for a pathogen and the limited ability of the biomarker to track all stages of the pathogen's life cycle, were strongly implicated in our study. Although we were unable to experimentally rule out the association between negative mRDT samples and genetic heterogeneity of HRP2 vs HRP3 genes as implicated in a number of studies across the globe the travel and exposure history associated with the study population, strongly suggest the circulation of non-falciparum species of plasmodium among the clinical cases [28]. Although the study site is a Military Hospital open to public, the attendees were mostly military personnel who have had extensive history of travelling on international peacekeeping missions in malaria endemic regions and their families. Therefore, they may have been exposed to non-falciparum species of Plasmodium, which escaped detection using the candidate mRDTs, which are pfhrp2-based and specific for only P.falciparum [29]. Indeed, using microscopy, non-falciparum species of Plasmodium (P) including P. malariae, P. ovale, and P. vivax were detected in 82% of samples that were negative for malaria using candidate mRDTs.

Furthermore, although we could not experimentally verify the association between our negative samples and the factor of low parasite density in this study, the remaining 18% of samples, which were negative using the candidate mRDTs, could be due to a lower parasite antigen titer in the blood at the time of sampling. Low parasite density was known to be associated with early infection and/or self-medication, which is fast becoming a common practice in Africa [30]. However, even though the candidate mRDTs showed a higher specificity to detect true negative cases, poor sensitivity metrics render them unreliable in the detection of malaria cases. The utilization of ecologically incentive mRDTs is detrimental to managing malaria among at-risk populations including children under five years of age, pregnant women and our military personnel exposed to non-falciparum species of Plasmodium. Consequently, this will compromise global health strategies to eradicate epidemic malaria by 2030.

4.1. Limitations

We were unable to overcome the inherent limitations associated with the pilot study. These included lack of funding to extend the study period to cover both raining and non-raining transmission seasons of malaria. The study covers only raining season when the transmission of malaria is known to be very high. In addition, our inability to use molecular method such as Polymerase Chain Reaction alongside the microscopy as gold standard, constituted a limitation to quality controlled the expert microscopy in this study. Therefore, the interpretation of our result should, therefore, be done within the context of our study design and settings.

5. Conclusions

Both mRDTs showed lower discriminatory accuracy compared to microscopy in this study. The observed variation between the metrics of 'external validity' and 'ecologic validity' has public Health policy and Research implications.

Recommendation

Due to the emergency and prevalence of non-falciparum species of plasmodium in different ecologies, Ghana should as matter of urgency accelerate and strengthen the implementation of the WHO's recommendation to switch from mono- biomarker based mRDTs to multiple- biomarker based mRDTs for confirmatory malariology. Additionally, it is essential to maintain a regular workshop for medical store managers at the regional and district levels to review the quality control mechanism on transport, storage and handling of RDT kits. Furthermore, it is crucial to conduct further research to consolidate our understanding on ecological risk factors, which could affect the validity and reliability of mRDT in primary health care centers in Ghana. The National Malaria Elimination Programme (NMEP) should refine strategy to contain our 'military population' as an emerging reservoir for the non-falciparum plasmodium parasite in Ghana. Additionally, the NMEP and health training institutions must intensify the diagnostic capacities of health workers, using diversified mRDTs.

References

1. Rowe, A. K. (2017). Assessing the health impact of malaria control interventions in the mdg/sustainable development goal era: a new generation of impact evaluations. The American journal of tropical medicine and hygiene, 97(3 Suppl), 6.
2. Who. (2020). World malaria report 2020: 20 years of global progress and challenges. World malaria report 2020: 20 years of global progress and challenges, 299.
3. Ghana Malaria Indicator. (2022). Trends:Out puts from a DHS Program Workshop, 2018.
4. Ghana: number of malaria cases. (2022).
5. D'adhemar J. (2020). Why eradicating Malaria in Africa requires a specific approach and new tools? Target Malaria.
6. Owusu-Agyei, S., Asante, K. P., Adjuik, M., Adjei, G., Awini, E., Adams, M., ... & Chandramohan, D. (2009). Epidemiology of malaria in the forest-savanna transitional zone of Ghana. Malaria journal, 8(1), 1-10.
7. 7. Odei-Lartey, E. O., Prah, R. K. D., Anane, E. A., Danwonno, H., Gyaase, S., Oppong, F. B., ... & Asante, K. P. (2020). Utilization of the national cluster of district health information system for health service decision-making at the district, sub-district and community levels in selected districts of the Brong Ahafo region in Ghana. BMC health services research, 20(1), 1-15.
8. Gmanyami, J. M., Ameko, A., Ahiafe, S. S., Bosoka, S. A., Kweku, M., & Ansah, E. K. (2020). Effect of pre-consultation testing on clinicians' adherence to malaria test results and waiting time among children under 5 years in the Northern Zone of Volta Region of Ghana. Malaria Journal, 19(1), 1-9.
9. Rosenthal, P. J. (2012). How do we best diagnose malaria in Africa?. The American journal of tropical medicine and hygiene, 86(2), 192.
10. Gatton, M. L., Dunn, J., Chaudhry, A., Ciketic, S., Cunningham, J., & Cheng, Q. (2017). Implications of parasites lacking Plasmodium falciparum histidine-rich protein 2 on malaria morbidity and control when rapid diagnostic tests are used for diagnosis. The Journal of infectious diseases, 215(7), 1156-1166.
11. WHO. False-negative RDT results and implications of new reports of P.falciparum histidine-rich protein 2/3 gene deletions. Geneva, World Health Organization.WHO/ HTM/201718.2017.
12. Ho Municipal (Municipal District, Ghana) - Population Statistics, Charts, Map and Location [Internet]. [cited 2022 Dec 26].
13. Volta Military Hospital, Volta, Ghana [Internet]. [cited 2022 Dec 26].
14. Chuks J Mba and Irene K.Aboh. (2006). Prevalence and management of Malria in Ghana: A case study of Volta Region; 2006, African Population Studies Vol.22 n°1/Etude de la population africaine vol. 22 n° 1.
15. UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, Centers for Disease Control (U.S.), Foundation for Innovative New Diagnostics. Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: round 4 (2012). Geneva: World Health Organization; 2012 [cited 2022 Dec 26] .
16. JavaStat -- 2-way Contingency Table Analysis. [cited 2022 Dec 26] .
17. Ghana malaria scorecard - quarter 2, 2020 [Internet]. ALMA Scorecard Hub. [cited 2022 Dec 27].
18. Adu-Gyasi, D., Asante, K. P., Amoako, S., Amoako, N., Ankrah, L., Dosoo, D., ... & Owusu-Agyei, S. (2018). Assessing the performance of only HRP2 and HRP2 with pLDH based rapid diagnostic tests for the diagnosis of malaria in middle Ghana, Africa. PloS one, 13(9), e0203524.
19. Abdulkadir, I., Rufai, H. A., Ochapa, S. O., Malam, M. S., Garba, B. I., Oloko, A. G. Y., & George, I. I. (2015). Malaria rapid diagnostic test in children: The Zamfara, Nigeria experience. Nigerian Medical Journal: Journal of the Nigeria Medical Association, 56(4), 278.
20. Koita, O. A., Sangaré, L., Sango, H. A., Dao, S., Keita, N., Maiga, M., ... & Krogstad, D. J. (2012). Effect of seasonality and ecological factors on the prevalence of the four malaria parasite species in northern Mali. Journal of Tropical Medicine, 2012.
21. World Health Organization. Roll Back Malaria, Development USA for I. New perspectives : malaria diagnosis : report of a joint WHO/USAID informal consultation, 25-27 October 1999 [Internet]. World Health Organization; 2000 [cited 2022 Dec 30]. Report No.: WHO/CDS/RBM/2000.14.
22. Dinko, B., Ayivor-Djanie, R., Abugri, J., Agboli, E., Kye-Duodu, G., Tagboto, S., ... & Awandare, G. A. (2016). Comparison of malaria diagnostic methods in four hospitals in the Volta region of Ghana. Malaria World Journal, 7(5), 1-6.
23. Prah, J. K., Amoah, S., Yartey, A. N., Ampofo-Asiama, A., & Ameyaw, E. O. (2021). Assessment of malaria diagnostic methods and treatments at a Ghanaian health facility. Pan African Medical Journal, 39(1).
24. Rogier, E., McCaffery, J. N., Nace, D., Svigel, S. S., Assefa, A., Hwang, J., ... & Halsey, E. S. (2022). Plasmodium falciparum pfhrp2 and pfhrp3 gene deletions from persons with symptomatic malaria infection in Ethiopia, Kenya, Madagascar, and Rwanda. Emerging Infectious Diseases, 28(3), 608.
25. King, M., George, A. E., Cisteró, P., Tarr-Attia, C. K., Arregui, B., Omeonga, S., ... & Mayor, A. (2021). No evidence of false-negative Plasmodium falciparum rapid diagnostic results in Monrovia, Liberia. Malaria Journal, 20(1), 238.
26. Mudare, N., Matsena-Zingoni, Z., Makuwaza, A., Mamini, E., Munyati, S. S., Gwanzura, L., ... & Mharakurwa, S. (2021). Detecting Plasmodium falciparum in community surveys: a comparison of Paracheck Pf® Test and ICT Malaria Pf® Cassette Test to polymerase chain reaction in Mutasa District, Zimbabwe. Malaria journal, 20(1), 1-7.
27. Miller, E., & Sikes, H. D. (2015). Addressing barriers to the development and adoption of rapid diagnostic tests in global

health. Nanobiomedicine, 2(Godište 2015), 2-6.
28. Molina-de la Fuente, I., Pastor, A., Herrador, Z., Benito, A., & Berzosa, P. (2021). Impact of Plasmodium falciparum pfhrp2 and pfhrp3 gene deletions on malaria control worldwide: a systematic review and meta-analysis. Malaria Journal, 20(1), 1-25.
29. Teneza-Mora, N., Lumsden, J., & Villasante, E. (2015). A malaria vaccine for travelers and military personnel: Requirements and top candidates. Vaccine, 33(52), 7551-7558.
30. Anchinmane, V. T., & Shedge, R. T. (2010). A review of malaria diagnostic tools: microscopy and rapid diagnostic test. Asian Journal of Medical Sciences, 1(2), 75-79.