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Original Research Paper

Performance Evaluation of Laboratory Professionals on Malaria Microscopy in the Volta Region of Ghana

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ABSTRACT

Background: Every malaria case needs to be tracked in a surveillance system. This means that every case that returns for follow-up after treatment must be tested and confirmed by microscopy but not with Rapid Diagnostic Test (RDT). However, studies have shown that light microscopy has been neglected in control programmes and evidence suggests that field standards are commonly poor. This study evaluated the performance of laboratory professionals on malaria microscopy in the Volta Region. Methods: A cross-sectional study design was used among 15 participants working in 10 health facilities in the Volta Region. A standardized pre-validated slide panel and questionnaire were distributed to participants. The slide panel was made up of 10 positive and 10 negative blood films slides. Participants were asked to examine the slides and their readings were compared to the expert readings. Agreement in detecting malaria parasite between participants and expert were estimated using the kappa score. Results: A total of 15 participants with mean age of 38.6±14.4 years were included in the study. The overall sensitivity and specificity of the participants in detecting malaria parasites were 86% and 77%, respectively. The overall positive and negative predictive values were 73% and 88%, respectively. The overall percent agreement between participants and expert Microscopist in the detection of malaria parasites was 80% and the Kappa index was 0.61. Best performing participants received training within the past 12 months. However, there was an overall weak positive relationship between participants and the expert on malaria parasite quantification ($r=0.31$, $p<0.001$, $\alpha=0.05$). Conclusion: The overall performance of participants in the detection and quantification was moderate, even though, the overall sensitivity, specificity, positive and negative predictive value and percent agreement of participants, were not greater than or equal to 90%. Training is needed in order to improve the performance of Microscopists.

Key words: Malaria microscopy, Performance, Laboratory professionals, Volta Region, Ghana.

INTRODUCTION

Malaria remains a major public health problem and approximately 3.2 billion people, nearly half of the world's population are at risk of malaria. Most malaria cases and deaths occur in sub-Saharan Africa (SSA) (WHO, 2016). In Ghana, Plasmodium falciparum is the predominant malaria parasite (about 80-90%) causing severe morbidity and mortality particularly in children under five years of age and pregnant women (Malaria, 2013).

According to the Ghana Demographic and Health Survey (GDHS), the prevalence of malaria in children aged 6-59 months was 36% as measured by RDT and 25.5% as measured by analysis of blood smears via microscopy (GDHS, 2014).

An accurate, correct laboratory diagnosis is essential as false negatives can result in untreated malaria patients with potentially severe consequences, including death. False negatives can also significantly undermine both the clinical confidence in laboratory results and credibility in the community (WHO, 2009).

Malaria Microscopy Quality Assurance Manual Version one the detection of malaria parasites by light microscopy is still the primary method of malaria diagnosis in health clinics and hospitals throughout the world. This requires a reliable microscopic service that is cost-effective, accurate and timely, and results have a direct impact on the treatment given to a patient. The effectiveness of malaria microscopy depends on maintaining a high level of staff competence and performance at all levels. Early diagnosis of malaria is a basis for the management of malaria, and the key to reducing malaria-related mortality and morbidity. Demonstration of the presence of malaria parasites under microscopy prior to treatment with anti-malarial drugs is fundamental to this goal since clinical diagnosis has a poor accuracy and leads to over diagnosis and increases the risk of anti-malarial drug resistance (Ayalew, 2014).

Reports from Ethiopia showed that 14 (19.4%) out of 72 participants were able to report correctly on all ten slides distributed whilst the rest 58 (80.6%) missed at least one slide. It was reported that sensitivity and specificity of participants in the detection of malaria parasites were 82% and 96.5%, respectively. Agreement between participants 88% and Kappa was 0.76 (Ayalew, 2014). Another study by Alemu in North Gondar, Canada also reported sensitivity and specificity to be 82.0% and 93.8%, respectively. Positive predictive and negative predictive were found to be 97.3% and 65.8%, respectively (Alemu, 2014).

In the Democratic Republic of Congo it was found that correct/acceptable scores for at least 4/5 slides was higher among participants with experience than those were assessed for the first time (40.9% versus 22.4%, $p = 0.001$) and higher among those who have been trained < 2 years before, compared to those who were not (42.9% versus 26.3%, $p = 0.01$) (Mukadi *et al.,* 2016). Report from a study conducted in 12 health centers in Kinshasa city found that, sensitivity for Optimal-ITW, Paracheck-PFW and microscopy was 79.7% and 86.2% and specificity was 97.0% and 49.1%, respectively and it was concluded that, although microscopy is considered as the "gold standard" for malaria diagnosis at the point-of-care level, its accuracy may not always be satisfactory when performed in health centers (Ashraf *et al.,* 2012).

The WHO African Region has had the largest increase in levels of malaria diagnostic testing, from 36% of suspected malaria cases tested in 2005 to 41% in 2010. This is due to an increase in the use of RDTs, which accounts for 71% of diagnostic testing among suspected cases. This implies that most African countries are almost on the verge of neglecting the use of microscopy which is the gold standard for detection of malaria and has rather accepted RDTs for testing of suspecting malaria cases. What happens if RDTs fail? Are our laboratory professionals and the various institutional laboratories equipped enough to detect malaria parasite using microscopy? Although light microscopy, established over 100 years ago and frequently considered the reference standard for clinical diagnosis, has been neglected in control programs, evidence suggests that field standards are commonly poor (Thiam *et al.,* 2011).

WHO recommended another malaria control measure based on the Test, Treat and Track (T3) strategy. This strategy recommends that; every suspected malaria case must be confirmed by microscopy or a rapid diagnostic test (RDT) before treatment is given with antimalarial drugs. This is to help reduce the emergence and spread of drug resistance by reserving antimalarial drugs for only those who actually have the disease. To ensure effective control, every malaria case should be tracked in a surveillance system. Ability to Test, Treat and Track will enable malaria elimination to be seriously anticipated (Thiam *et al.,* 2011). During the tracking or follow-up period, only microscopy can be used to test for malaria. This is because RDT can still test positive for someone whose malaria parasites have recently been cleared.

In 2013, Ghana subscribed to the WHO's recommendation and developed guidelines for the implementation of the T3 strategy by updating the 2009 malaria case management guidelines. Health care providers at various levels of the health system received training on the T3 strategy for the new malaria case management. Since the implementation of the T3, the Ghana Health Service (GHS) report shows that there has been an increased in the proportion of OPD malaria cases tested by microscopy or RDT from 48% in 2013 to 73.5% in 2014, representing a 53% increase. This is the best performance in four years with 73.5% of all OPD malaria cases tested before treatment was given (GDHS, 2014). In addition, data from the district health information system II, (DHIMS2) show that the testing rate of suspected cases of malaria in the Volta region has

increased from 23% in 2011 to 41% in 2015 (DHIMS2, 2016).

Although there has been an increase in the testing rate of suspected malaria cases in the region, there is no evidence to show the number of cases that were tracked using microscopy as well as, the quality of the tests performed. Recent observations in Ho Municipality (Kpetengyne *et al.,* 2016) and Bongo district (Aganda *et al.,* 2016) have shown that health facilities were not able to complete the T3 strategy and the determining factor was tracking. Reasons for not completing T3 strategy were that there were no facilities for diagnosing if asked to return for follow-up and sometimes no personnel available to do the test. The current study, therefore, evaluated the performance of laboratory professionals on malaria microscopy (Kpetengyne *et al.,* 2016), (Aganda *et al.,* 2016).

MATERIALS AND METHODS

Study area

This study was conducted in the Volta region of Ghana which is one of the 10 administrative regions located at the eastern part of the country. The Northern Region bounds it to the north, the south by the Gulf of Guinea, west by the Volta Lake and the east by the Republic of Togo. The region occupies a surface area of about 20,570 square kilometers representing 8.6% of the total land mass of Ghana. It lies between latitudes 5o 45'N and 8o 45'N and it is divided into 25 administrative Municipal and Districts. Based on the 2010 National Population and Housing Census, the population of the Region was 2,396,397 with an annual average growth rate of 2.4% (Volta Regional Health Directorate, 2016).

The region is divided into three geographical zones namely; the northern zone, middle zone and the southern zone. The middle and northern zones are mainly mountainous where the highest peak in the country, Mount Afadzato (885m) is located. The southern zone is flat with marshy and sandy portions. This coastal area has low-lying altitude from less than 15 metres above sea level. The main economic activities of the people include agriculture, fishing, handicrafts, the forestry industry and salt mining. The region has 581 health facilities, providing various types of services at all levels. Out of these 581 health facilities, there are 334 Community-Based Health Planning Services (CHPS) compounds, 40 clinics, 6 maternity homes, 3 polyclinics, 31 hospitals of which 17 are district hospitals, 1 regional hospital and 11 other hospitals. Out of the 31 hospitals, 6 are in the northern zone, 8 in the central zone and 17 in the southern zone. In addition, there are 159 health centers in the region of which 36 are in the northern zone, 78 in the central zone and 52 in the southern zone. The predominant species of malaria parasite in Ghana is P. *falciparum* [Malaria, 2013, GDH, 2014]. *P. vivax, P. malaria* and *P. ovale* are not locally dominant.

Study population

The study population was all laboratory professionals who were working on malaria microscopy in health facilities in the Volta region.

Inclusion and Exclusion Criteria

Inclusion criteria

All malaria Microscopists who were working at the selected health facilities in the region were included in the study.

Exclusion criteria

All Laboratory professionals who were not working on malaria microscopy and those who were working on malaria microscopy but did not give informed consent were not included in the study.

Study Design

This was a cross-sectional study design which involved ten (10) health facilities. Five (5) health centers, four (4) hospitals and a university laboratory. From a list of all hospitals and health centers, facilities were selected by ballot. At the selected facility at least one laboratory professional was randomly selected for the study. Twenty prepared blood film slides from the research centre were given to each participant to examine.

Sample size determination

Based on the Informal Consultation on Quality Control of Malaria Microscopy, the recommended number of malaria blood film slides set for assessment by the malaria Microscopist was 20 minimum. A total of 300 malaria blood films from the research centre was used for the study. Fifteen participants (malaria Microscopist) from the 10 randomly selected health facilities were used for the study.

Sampling procedure for the participants

The study area population was divided into three zones (northern zone, the central zone and the southern zone). A sample sampling frame from each zone containing a list of all the hospitals and health centers in that zone with at least one malaria Microscopist available was used. The facilities were randomly selected from each sample sampling frame in each zone using simple random sampling method (balloting). The samples were selected by writing down all the names of the hospitals and health centers in that zone on sheets of paper into a container. A neutral person who was not part of the study was asked to draw from the container to select two hospitals and two health centers in that zone. Thus, simple random sampling (balloting) was used to select at least one

Microscopist from the selected hospitals and health centers in each zone.

Data collection Procedure

Slide Panel Characteristics

The malaria blood film slide set was made up of 10 positive blood film slides with different densities (low and high densities), and 10 negatives. The slides were prepared based on the locally prevalence species in the Volta region which were mainly P. falciparum and few slides of *P. malariae*. To standardize the procedure, a 20-panel slide was used for the assessment. This was based on the recommendation from the Informal Consultation on Quality Control of Malaria Microscopy [Cohen, 1960]. The panel slide was composed of:
10 negatives malaria blood film slides,

4 slides of *P. falciparum* with a density of 50-100 mm^3

parasite/ ,
4 slides of *P. falciparum* with a density of 300-500 mm^3

parasite/ ,
2 slides of *P. malaria* with density of >500 parasites/ mm^3

Administration of Panel Slide

Twenty (20) Giemsa-stained thin and thick blood films with questionnaires were delivered to all the malaria Microscopists working at the 10 selected facilities in the region. Two malaria microscopy Experts read and interpreted the blood smears using two diagnostic criteria: 1) the presence or absence of malaria parasites and, 2) Quantification of the parasitic load for each species. “Load” was defined as the number of parasites per 200 white blood cells in high power thick fields and multiplied by a standard multiplier of 8,000 WBC/µl of blood.

Counting parasites (quantitation)

For accurate parasite density estimation based on parasites per microliter or white blood cell (WBC) count, it was recommended in routine practice that parasite quantification is performed against 200 or 500 WBCs. If, after counting 200 WBC, 100 or more parasites are found, the results were recorded in terms of a number of parasites/200 WBC. If less than 100 parasites were found after counting 200 WBCs, parasite quantification would be continued until 500 WBCs were counted. (This gave a probability <5% of chance variation greater than 25% of true parasite density using a x100 oil immersion objective and an eyepiece with a field number of 18). All parasites in the final field were counted even if the count exceeded 500 WBCs. To determine parasite density, the parasite count was adjusted against the true WBC, where available. If unavailable, the common practice was to assume a WBC of 8000/µL (Table 1).

Accuracy of parasite detection and species identification

According to the quality control of malaria microscopy interim grading system for accreditation of malaria Microscopist, for accurate parasite detection and identification of species, the acceptable level of agreement for “Expert” and “Reference” Readers or Microscopists was ≥90% and ≥80%<90% respectively, while, ≥70%<80% and<70% was considered accurate identification of species for “Advanced” and “i- training” Readers or Microscopists [Cohen, 1960].

A total of 200 minutes (10 minutes per BF slide) was allocated for each Microscopist to read the BF slides [Cohen, 1960].

Data analysis

Data were collected and entered into Epi-data version 3.1 and exported to Stata version 14 for analysis. The level of agreement in malaria parasite detections, species identification and quantification of malaria parasite was compared with independent variables. Association was considered significant at $p<0.05$. False positive rate (% false positives) and false negative rate (% false negatives) were calculated to see the discordant results. Chi-square (for categorical data), sensitivity, specificity, percent agreement, and kappa score were calculated to assess participants’ performances in parasite detection and identification of Plasmodium species using light microscopy. Based on WHO recommendation, participants were classified as: “In training”- when the agreement with the expert Readers in malaria parasite detection and species identification was less than 70%; “Advanced”- when the agreement was greater than or equal to 70% but less than 80; “Reference”-when the agreement was greater than or equal to 80% but less 90%; and “Expert” -when the agreement was greater than or equal to 90% [Cohen, 1960]. Kappa Value was also calculated (Kappa, K=((po-pe))/((1-pe))) [Viera and Garrett, 2005], to see the strength of an agreement. Based on the calculation, the strength was classified as: <0.20 Slight agreement, 0.21–0.40 Fair agreement, 0.41–0.60 Moderate agreement, 0.61–0.80 Substantial agreement and 0.81–0.99 Almost perfect agreement [Clendennen *et al.,* 1995]. Pearson product moment correlation coefficient was computed to measure the strength and direction of the relationship between participants and expert Reader on parasite quantification. Based on the correlation coefficient, the strength of agreement between participants and expert was classified as: <0.19 a very

Table 1. Interim WHO grades for accreditation for Malaria Microscopist..

Interim grades for final competency assessment for Microscopist			
Grade	**Accuracy of species identification**	**Parasite quantification**	**Parasite detection**
1. Expert	≥90%	≥50%	≥90%
2. Reference	80%-<90%	≥40%	80%-<90%
3. Advanced	70%-<80%	≥30%	70%-<80%
4. In training	<70%	<30%	<70%

weak correlation which meant a weak agreement, 0.20-0.39 a weak correlation which meant weak agreement, 0.40-0.69-moderate or good agreement, and 0.70-0.89 – a strong agreement and 0.9-1.0- a very strong agreement. However, when the correlation coefficient was negative it meant that participants and expert were not in agreement in parasite quantification.

Ethical issues

Ethical approval for the study was obtained from the Ghana Health Service (GHS) Ethical Review Committee (ERC) with approval number (GHS-ERC: 16/02/2017). Permission to conduct the study was sought from the Volta Regional Health Directorate and the health facilities selected for the study. Confidentiality and anonymity were assured by using identification codes instead of names in data analysis and reporting process. An Informed consent form which provided details and willingness to participate in the study was provided and made clear to the participants. The content of the study was fully disclosed to participants and their consent was sought after disclosure. Although there were no known risks associated with the research protocols, participants who felt uncomfortable had the right to opt out. Data was stored under lock and key and was directly accessible to only personnel (thus, the principal investigator and supervisor) directly involved in the study.

RESULTS

Table 2 shows a comparison of sample estimates and agreement between Readers (participants) and an expert Reader. Out of the 15 participants surveyed, the overall sensitivity and specificity in detecting malaria parasite were 86% and 77%, respectively. The overall positive predictive value and negative predictive value was 72.6% and 88%, respectively. The overall percentage agreement in detecting malaria parasite was 80%, while the overall kappa was 0.61, which implies moderate agreement.

Out of the 15 participants, 7 (46.7%) had 100% sensitivity, 5(33.3%) had a sensitivity greater than or equal to 80 but less 90% (≥ 80% <90%). The rest had sensitivity below 70%. However, only 2(13.3%) had a specificity above 90%, the majority 6(40.0%) had a specificity greater than or equal 70 but less than or equal to 80% (≥ 70% ≤80%). Five (33.3%) had a specificity above 80 but less than or equal to 90% (> 80%≤90%). The rest (13.3%) had below 70% specificity.

Only 2(13.3%) of the 15 participants had 90% positive predictive value, 9(60%) had a positive predictive value greater than 70 but less than 80% (≥ 70% <80%). The rest 4(26.7%) had a positive predictive value <70%. However, of the 15 participants surveyed, 7 (46.7%) had 100% negative predictive value, while, 6(40.0%) had a negative predictive value greater than or equal to 80 but less than 90% (≥ 80% <90%). The rest had a negative predictive value < 70%.

Of the 15 participants, 4(26.7%) had a percentage agreement above or equal to 90% with the expert Reader, 6(40.0%) had a percentage agreement greater than or equal to 80 but less than 90% (≥ 80% <90%). Only 2(13.3%) had a percentage agreement greater than or equal to 70 but less than 80% (≥ 70% <80%). The rest had a percentage agreement less than 70%.

Table 2 also shows that Readers 1 (kappa=0.8), 6 (kappa=0.9) and 12 (kappa=0.89) had an almost perfect percentage agreement with the expert Reader in malaria parasite detection, Readers 15 had a slight agreement with the expert Reader (kappa=0.1). Figure 1 shows that out of the 15 participants surveyed, 4(26.7%) were rated as “Expert” based on the WHO grading system. The majority 6(40.0%) of the participants were rated as “Referenced” and 2(13.3%) as “Advanced”. Only 3(20.0%) were rated as “In-training”. Table 3 shows that out of 300 blood film (BF) slides distributed for examination, only 296(98.7%) were examined. The participants and the expert agreed on a result of 106 positive slides (true positives) and 132 negatives (true negatives). However, the participants and the expert disagreed on 18 slides with positive readings (false positives) and 40 slides with negative readings (false negatives). False positive rate (percentage of negative BF slides reported as positive) and false negative rate (percentage of positive slides reported as negative) were 14.5% and 23.3%, respectively. Out of the 296 BF slides read by the participants, 139(47.0%) were read by participants from the health center. The false positive and false negative rates were 13.8% and 23.5% respectively for participants from the health center. The sensitivity and specificity for participants from the health center were 86.2% and 76.5%, respectively.

Table 2. Comparison of sample estimates and agreement between Readers and an expert Reader.

Readers	Number of slides Examined	Prevalence Pr(A) 95(CI) (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (PPV) (%)	Negative predictive value (NPV) (%)	Percent agreement (PA) (%)	Positive percent agreement (PPA) (%)	Kappa index of the individual vs the expert reading
Reader1	20	40(19,63.9)	100	83	80	100	90	80	0.8
Reader2	20	55(32,76.9)	82	89	90	80	85	90	0.7
Reader3	20	37(16,61.6)	86	75	67	90	79	67	0.56
Reader4	20	55(32,76.9)	64	67	70	60	65	70	0.3
Reader5	20	45(23,68.5)	89	82	80	90	85	80	0.7
Reader6	20	45(23, 68.5)	100	91	90	100	95	90	0.9
Reader7	20	65(41,84.6)	62	71	80	50	65	20	0.3
Reader8	20	50(27,72.8)	80	80	80	80	80	80	0.6
Reader9	20	35(15,59.2)	100	77	70	100	85	70	0.7
Reader10	20	50(27,72.8)	80	80	80	80	80	80	0.6
Reader11	20	40(19,63.9)	100	83	80	100	90	80	0.8
Reader12	20	39(17,64.3)	100	91	88	100	94	88	0.89
Reader13	20	30(12,54.3)	100	71	60	100	80	60	0.6
Reader14	20	26(9.1,51.2)	100	71	56	100	79	56	0.57
Reader15	20	15(3.2,37.9)	67	53	20	90	55	20	0.1
Overall	**300**	**42(36,47.7)**	**86**	**77**	**73**	**88**	**80**	**73**	**0.6**

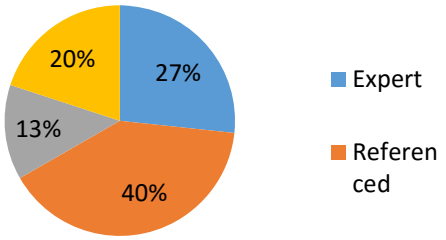

Figure 1. Level of agreement on detection of presence or absence of malaria parasite based on WHO grading system.

Percentage agreement of participants from a health center was 80.6% (kappa=0.61) which is 'substantial agreement'. The School of Public Health (SPH) laboratory read the least number of slides 40 (14%). They reported a very high false positive rate and false negative rate, 29.2% and 18.8% respectively as compared to those from the health centre and hospital. They reported a low sensitivity and specificity, 70.8% and 81.3% respectively as compared to those from the health centre and hospital. Percentage agreement of participants from the SPH Laboratory was 75% (kappa=0.50) which is rated as 'moderate agreement'. Table 4 shows that out of 150 BF slides for quantification, only 92(62.3%) were correctly identified for quantification. There was an overall weak positive relationship between participants and the expert on malaria parasite quantification and this was statistically significant ($r = 0.31$, $p<0.001$, $\alpha=0.05$). Table 4 also shows that only 3(20.0%) of the Readers had a strong positive linear relationship with the expert Reader and this was statistically significant since their correlation coefficients (r) were greater than 0.80 and p values<0.05. Two (Reader 11 and 12) out of the three participants that had a strong positive relationship attained BSc in biomedical sciences, while one attained middle school. All the three participants had malaria in-service training as at the time of the survey. Two out of the three had above 5 years work experience in malaria microscopy. Two out of the three examined between 20 and 40 BF slides per day, while one examined above 40 BF slides per day. The majority 5(33.3%) of the Readers had a moderate positive linear relationship with the expert Reader and this was statistically significant since their correlation coefficients (r) fall within the range 0.50-.70 and p values <0.05.

Of the 5 that had a moderate positive relationship, three had attained diploma, while one attained BSc and one attained middle school level. Four out of 5 Readers had less than 5 years' experience and had participated in in-service training as at the time of the survey. All the five participants examined 20-40 BF slides per day.

Three (20.0%) of the Readers had a weak positive linear relationship ($0\geq r<5$) with the expert Reader and this was not statistically significant since p value >0.05 Two out of the three participants that had a weak positive relationship had less than 5 years working experience. One had no in-service training, however, two had above 5 years working experience. Two of the Readers examined above 40 BF slides per day, while one examined less than 20 BF slides per day.

There was a very weak negative linear relationship between one participant and the expert but this was not statistically significant ($r = -0.01$, $p=0.973$, $\alpha=0.05$). This participant had a low level of education (JHS), less than 5 years working experience and had no in-service training in malaria microscopy and was from a health centre. This reader read less than 20 slides per day.

DISCUSSION

The introduction of the Test, Treat and Track (T3) initiative in 2010 by the WHO recommends that every suspected malaria case must first be confirmed by RDT or microscopy. To ensure effective control, every malaria case needs to be tracked in a surveillance system. To track or follow-up means that every case that returns for follow-up must be tested and confirmed by microscopy but not with RDT. However, studies have shown that light microscopy which is the preferred standard for confirming suspected malaria cases in health clinics and hospitals throughout the world has been neglected in control programs and evidence suggests that field standards are commonly poor. The effectiveness of malaria microscopy depends on maintaining a high level of staff competence and performance at all levels (WHO, 2009). This current study assessed the performance of malaria Microscopists in the Volta Region.

The overall agreement kappa value on detection of malaria parasites with expert Reader was 0.6, which was defined as 'moderate agreement' based on the Kappa index interpretation (Clendennen *et al.,* 1995). The overall agreement kappa value in the current study was in agreement with what was reported by other authors, who also found Kappa = 0.61 [16]. However, this current study had a lower Kappa than what was reported in

Table 3. Overall Sensitivity, Specificity and percentage Agreement on detection of presence or absence of malaria parasite between participants and expert Reader and type of facility.

Variable	Participant Reader	Expert Reader			Sensitivity (%)	Specificity (%)	Percent Agreement	Kappa	Odd ratio 95%(CI)
Facility Type									
		Positive	Negative	Total					
Health Centre	Positive	50	8	58	86.2	76.5	80.6	0.61	20.4(8.33,49.8)
	Negative	19	62	81					
	Total	69	70	139					
Hospital	Positive	39	3	42	92.9	76.0	82	0.64	41.2(11.9,140)
	Negative	18	57	75					
	Total	57	60	117					
SPH laboratory	Positive	17	7	24	70.8	81.3	75	0.50	10.5(2.38,45.6)
	Negative	3	13	16					
	Total	20	20	40					
Overall	Positive	106	18	124	86	77	80.41	0.61	19.4(10.6,35.7)
	Negative	40	132	172					
	Total	146	150	296					

Table 4. Correlation between participant Readers and Expert on Parasite quantification and their educational level, work experience, in-service training and facility type.

Participants Readers	Number of positive slide For quantification	Number of positive slide correctly identified For quantification n (%)	product moment of Pearson correlation coefficient r*	P-value	Level of education	Level of facility	Work experience (years)	Training within past 2 years	Number of slides examined per day (Work load)
Reader1	10	8(80)	0.8133*	<0.001	JHS/Mid	HC	>5	Yes	20-40
Reader2	10	9(90)	0.6968*	<0.001	Diploma	SPH	<5	Yes	20-40
Reader3	10	-	-.	.	JHS	HC	>5	Yes	20-40
Reader4	10	7(70)	0.2707	0.248	JHS	HC	>5	Yes	40 and above
Reader5	10	8(80)	0.2037	0.389	Diploma	HC	<5	No	40 and above
Reader6	10	9(90)	0.6638**	<0.001	BSc	H	<5	No	20-40
Reader7	10	8(80)	0.2917	0.212	BSc	SPH	<5	Yes	<20
Reader8	10	7(70)	0.5299**	0.020	JHS	HC	>5	Yes	20-40
Reader9	10	7(70)	-0.0081	0.973	JHS	HC	<5	No	<20
Reader10	10	8(80)	0.5690**	0.009	Diploma	H	<5	Yes	20-40
Reader11	10	8(80)	0.8160*	<0.001	BSc	H	<5	Yes	40 and above
Reader12	10	6(60)	0.8811*	<0.001	BSc	H	>5	Yes	20-40
Reader13	10	-	-.	.	JHS	HC	>5	Yes	<20
Reader14	10	5(50)	0.5881**	0.008	Diploma	H	<5	Yes	20-40
Reader15	10	2(20)	0.1346	0.572	BSc	H	>5	No	<20
Overall	150	92(61.3)	**0.3118***	**<0.001**					

* Pearson product moment correlation coefficient * p<0.001, **p=0.01,

Hawassa town, Ethiopia (Kappa=0.67), which was defined as 'substantial'(Ayalew *et al.,* 2014). In this current study, the kappa finding was, however, much higher than a study from four laboratories in Mpumalanga province, South Africa, which showed a level of disagreement (kappa =0.11) (Durrheim *et al.,* 1997).

Out of the 300 malaria blood film (BF) slides which was distributed for examination, 296(98.7%) were examined while 4(1.3%) were not. The participants and the expert agreed on the results of 106 positive slides (true positives) and 132 negatives (true negatives). However, the participants and the expert disagreed on 18 slides with positive readings (false positives) and 40 slides with negative readings (false negatives). In this current study, false positive rate (defined as percentage of negative BF slides reported as positive) was 14.5%. The false positive rate in this current study was higher than the 6.9% reported in Hawassa town (Ayalew *et al.,* 2014). It was, however, lower in the study conducted in Democratic Republic of Congo, which reported 24.6% false positive rate (Mukadi *et al.,* 2016) and in Zambia, 24.2% (Prescott *et al.,* 2012). False positive results suggest that participants often incorrectly reported the presence of parasites; this could cause other diseases to be overlooked and not treated in a timely manner; hence, contributes to an increase in non-malaria morbidity and mortality, the misuse of antimalarial drugs, the development of parasite drug resistance, increased costs to the health services and patient dissatisfaction in the Region.

The false negative rate (defined as percentage of positive slides reported as negative) in this current study was 23.3%. This finding was higher than the 16.3% reported in Democratic Republic of Congo (Mukadi *et al.,* 2016], the 4.1% in Zambia (Prescott *et al.,* 2012) and the 3% reported in the USA (Kachur *et al.,* 1998). False negatives could result in untreated malaria patients and potentially severe consequences, including death. It could also significantly undermine both the clinical confidence in laboratory results and credibility in the various health facilities in the region.

The overall sensitivity and specificity of participants in detecting malaria parasites were 86% and 77% respectively. The sensitivity of this current study was almost similar to what was reported in Kinshasa city 86.2%. It, however, reported a very high specificity than what was in Kinshasa city 49.1% (Ashraf *et al.,* 2012). This current study reported a higher sensitivity and a lower specificity when compared to what was reported in Hawassa town, Ethiopia 82% and 96.5% respectively (Ayalew *et al.,* 2014) and in Haiti 83.6% and 88.6% respectively (Agandaa *et al.,* 2016). However, it reported lower sensitivity and specificity when compared to the study in Zambia, 88% and 97%, respectively (Prescott *et al.,* 2012). The overall high sensitivity in this study on the detection of parasites suggests that there were few false negative results, which means that there were few misdiagnoses of true infections.

In this current study, the overall positive predictive value (defined as the proportion of blood film slides with a positive test result that was correctly identified) and negative predictive value (the proportion of blood film slides with a negative test result that was correctly identified) was 72.6% and 88% respectively. Both positive predictive value and negative predictive values in this current study were lower than what was reported in Zambia 76% and 96%, respectively (Prescott *et al.,*2012). The positive predictive value in this study was higher than what was reported in Kinshasa city, 27.3% and in Haiti, 22.2%. However, the specificity in this current study was lower that what was reported in Kinshasa city 92% (Ashraf *et al.*, 2012) and in Haiti, 99.3% (Agandaa *et al.,* 2016). The overall accuracy of participants in detecting malaria parasite was low. The negative predictive value was not greater than or equal to 90%, indicating that participants could not be confident that a negative blood slide result strongly suggests that malaria was not the cause of the patient's illness.

In the current study, the performance of participants in detecting malaria parasite from the hospital was better than those from the health centre. The sensitivity, specificity and percentage agreement of participants from the hospital were 92.9%, 76.0% and 82% respectively compared to 86.2%, 76.5% and 80.6% from the health centre respectively. However, both institutions had similar agreement kappa value. (Kappa=0.64) for the hospital and (kappa=0.61) for the health centre. Both facilities had the same rating 'substantial agreement' based on the Kappa index interpretation (Clendennen *et al.,*1995).

In this current study, even though statistically significant, there was an overall weak positive relationship between participants and the expert on malaria parasite quantification ($r=0.31$, $p<0.001$, $\alpha=0.05$). Weak positive correlation means that agreement between participants and expert Reader on malaria parasite quantification was weak or poor. The reason for this poor performance might be due to the fact that the majority 8(53.3%) of the participants had less than 5 years working experience. In addition, the majority, 11 out of 15 participants read above 20 slides per day. This might have prevented them from quantifying parasitaemia routinely hence the lack of experience. However, quantification of parasitaemia is important because it provides information for clinicians to make decisions in using more potent antimalarial drugs for treatment.

Only 3 (20.0%) of the participants had a strong positive linear and statistically significant relationship with the expert Reader (Table 4). A strong positive linear relationship means that agreement between the participants and expert Reader on parasite quantification was strong or high. Reasons for this very good performance might be due to the fact all the three participants had in-service training on malaria microscopy. Two out of the three had above 5 years work experience in malaria microscopy. Two examined between 20 and 40 blood film (BF) slides per day, while

one examined above 40 BF slides. Participants from the hospital performed better than those from the health centre in malaria parasite quantification. Two out of three participants that had a strong positive correlation with the expert in parasite quantification were from the hospital compared to one from the health centre. Also, three out of the five that had a moderate correlation with the expert were also from the hospital.

Based on WHO recommendation, the current study found 26.7% of the participants as 'experts' based on the level of agreement in detecting malaria parasite. This finding was slightly higher than the study conducted in Hawassa town which found 25% as experts. The current study found 20.0% of participants as 'in-training' based on the level of agreement. This finding was slightly lower than the study conducted in Hawassa town, which found 23.6% participants as 'in training' (Ayalew *et al.,* 2014). Participants with 'in training' level of agreement, indicates that they need more training to improve their competency in the detection of the malaria parasite. However, those with 'expert' level of agreement means that such participants have high level of accuracy in malaria parasite detection

Limitations of the study

The limitations of the current study were that it did not evaluate the performance of the laboratory personnel with regard to the preparation of smears and the staining of blood films for malaria diagnosis. Participants were not evaluated on species identification. The study was not able to include all the malaria Microscopists in the various facilities in the Region. This was due lack of enough time and financial resources to fund the study. The effect of the clustering nature of the Readers (participants) has not been taken into account in this analysis.

Conclusion

The participants had a low sensitivity and a relatively high specificity in the detection of malaria parasites. Agreement of the participants with expert Microscopist in the detection of malaria parasites was better than agreement in parasite quantification. The performance of the participants working at health centre laboratory and Hospital laboratory on malaria detection and quantification were very low. However, participants from the hospital performed better than those from the health centre. The overall percent agreement, agreement kappa value, sensitivity, specificity, positive and negative predictive values of the participants were not greater than or equal to 90%. However, the overall performance of the participants in the detection and quantification was moderate.

Recommendation

Based on the findings of this study, the following recommendations are made to ensure accurate laboratory diagnosis of malaria in the Volta region: The GHS the NMCP should work on the implementation of regular competency assessment and training policy in all the selected health facilities in the Region. Stakeholders of malaria control program should organize an in-service training for all malaria Microscopists on malaria parasite quantification, to improve their competencies. The Regional Health Directorate should also provide an adequate number of human resources to the various facilities in the Region to aid laboratory diagnosis of malaria. About 16% of the participants mentioned that commercialized immersion oil and stain stock are of low quality and affect microscopy results. Supervisors (the regional laboratory manager) should investigate this claim.

List of Abbreviations

ACT -Artemisinin Combination Therapy, BF- Blood Films, DHIMS - District Health Information Management System, EQA- External Quality Assurance, GHS -Ghana Demographic Health Survey, NMCP- National Malaria Control Programme, NPV- Negative Predictive Value, nPCR- Nested PCR, OPD- Out-patient Department, WBC- White Blood Cells, WCC- White Cell Count, RDTs- Rapid Diagnostic Test.

Competing interests

The authors declare that they have no competing interests.

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