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Performance of Quantitative Buffy Coat, QBC Fluorescence and Staining Technologies™ Test, and SD Bioline™ Malaria Rapid Test in Malaria Diagnosis in Western Kenya

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Abstract

Malaria remains the most important parasitic disease in sub-Saharan Africa as a cause of morbidity and mortality. Effective management of malaria relies on prompt and accurate diagnosis to guide treatment. The World Health Organization (WHO) recommends that all suspected malaria cases be tested before initiation of treatment, thus diagnosis of malaria requires parasitological confirmation of malaria parasites in the blood of suspected patients. This cross-sectional study conducted at the Ahero County Hospital, Kisumu, Kenya, evaluated the performance of quantitative buffy coat (QBC) (QBC Fluorescence and Staining Technologies™(QBC F.A.S.T.™)-improved QBC system and SD Bioline™ malaria rapid tests) against 'gold standard' (Giemsa blood stained slides microscopy) for the detection of Plasmodium species in children<five years old (n=385)in a malaria holo-endemic area of western Kenya. Real-time PCR was performed on discrepant samples across the tests and the gold standard (microscopy).Sensitivity of QBC, QBC F.A.S.T.™ and SD Bioline™ malaria rapid tests were 90% (95% CI: 85-94), 77% (95% CI: 71-83) and 91% (95% CI: 86-94), respectively, while specificity was 30% (95% CI: 24-37), 83% (95% CI: 77-88) and 67% (95% CI: 60-73), respectively. The positive predictive values (PPV) were 58% (95% CI: 52-63), 83% (95% CI: 77-88) and 74% (95% CI: 63-80), respectively, while the negative predictive values (NPV) were 74% (95% CI: 63-84), 78% (95% CI: 71-83) and 87% (95% CI: 81-92), respectively.Although the standard QBC malaria test and the SD Bioline™ malaria rapid diagnostic test (RDT) showed better sensitivity relative to the improved QBC F.A.S.T.™ test, the latter had a better specificity. The performance of these tests remains modest against microscopy.

Keywords: Malaria, Quantitative buffy coat, QBC F.A.S.T.™, SD Bioline™

1. Introduction

People living in more than 97 countries around the world remain at risk of malaria, with 198 million cases and about584,000 of deaths reported in 2013 (WHO 2014). Over 80% of these deaths occur in sub-Saharan Africa (WHO 2011). Malaria diagnosis and prompt treatment with artemisinin combination therapy (ACT) remains the mainstay of malaria control in Africa (Gosling 2008). Parasitological diagnosis is the diagnostic cornerstone of choice since malaria symptoms and signs overlap with other febrile diseases (White 2005). The World Health Organization (WHO)currently recommends that all suspected malaria cases be parasitologically-confirmed before initiating treatment (WHO 2010) and this has been adopted by many countries, including Kenya. By 2013, the WHO African Region reported the largest increase in the number of suspected cases being tested (62%) compared to 47% in 2010 when this was initiated (WHO 2014).

Confirmatory malaria infection requires the availability of affordable, rapid, sensitive, and specific tests. Currently, the malaria diagnosis 'gold standard' method remains light microscopy with a variable limit of detection of 20-40 parasites/µL in good hands (Schindler *et al.* 2001). Microscopy also allows estimation of parasite density. However, the technique is human dependant, labour intensive requiring well-trained microscopists for reliable accurate results. Microscopy is not readily deployable in remote areas that lack equipped laboratories (Pinto *et al.* 2001). Due to these limitations there has been an increase in development of alternative diagnostic tools. A number tools, such as quantitative buffy coat (standard QBC) test and immunochromatographic tests such as rapid diagnostic tests (RDTs) have been developed and deployed for routine to complement microscopy. These methods have been found to be easier to use, sensitive and accurate (Bhandari *et al.* 2008)(RDT evaluation programme) but these methods detect malaria antigens in blood. Most RDTs available on the market target *Plasmodium falciparum*-specific,histidine-rich protein II (HRP-2) and *Plasmodium*lactate dehydrogenase enzyme (p-LDH). Some tests detect pan-specific pLDH or adolase from the parasite glycolytic pathway found in all *Plasmodium* species (Wongsrichanalai *et al.* 2007). A well-known

limitation of the RDT is the occurrence of false positive results caused by persistent antigenaemia even after effective anti-malarial treatment. This is peculiar to those tests that detect the parasite antigen HRP-2 specific to *P.falciparum* (Gitonga W. Caroline and Snow W. Robert 2012). More sensitive molecular techniques, such as polymerase chain reaction (PCR) and flow cytometry, have also been explored in laboratories to enhance detection of malaria parasites at very low parasite densities. These molecular methods remain costly, limiting their use to reference laboratories (Tangpukdee *et al.* 2009). All these techniques have their own limitations with respect to sensitivity, specificity, turnaround time, cost effectiveness, and ease of performance of procedures.

Even though there has been a rapid increase the use of RDTs globally, like other biological tests, malaria RDTs are temperature sensitive (Gitonga W C and Snow W R 2012) and performance alters in case of exposure to extremes of weather. There is a need to develop and deploy more robust methods. This study evaluated the performance of the QBC malaria test, QBC F.A.S.T.™test (improved QBC)and the SD Bioline™ malaria RDT against microscopy using Giemsa-stained blood slides for malaria diagnosis in young children aged <five years residing in a malaria holo-endemic area of western Kenya.

2. Methods

2.1 Study area

The study was conducted from May to September, 2013 at the Ahero County Hospital in Kisumu County, Kenya. The study area is situated in a rice irrigation scheme with high malaria transmission (Bukhari 2011). The hospital has antenatal and child health clinics from Mondays to Fridays from where potential study participants were screened and enrolled.

2.2 Study design

A hospital-based, cross-sectional study was conducted in children <five years of age at the Ahero County Hospital, Kisumu County, Kenya after fulfilling the inclusion criteria (children aged six-59 months presenting with fever and who had not taken any anti-malarial drugs within 14 days of reporting to the hospital,fever of ≥37.5°C). Individuals who had taken anti-malarial drugs within 14 days of reporting to the hospital were excluded from the study. The children were enrolled after parents/guardians provided a written informed consent obtained by research nurses and clinicians.

2.3 Ethical approval

The study was approved by National/Kenya Medical Research Institute Ethics Review Committee, Kenya (SSC# 2008).

2.4 Sample collection

Consecutive parents/guardians of patients aged six-59 months presenting at the study site were approached by study nurse/clinicians for recruitment. After consenting, capillary blood samples by finger-prick were collected into 0.5 mL microtainers (K_2EDTA-BD, USA) and processed within 2 hours of collection. The sample processing and conduct of the malaria diagnosis tests were performed at the study site by trained laboratory technicians. Prior to study initiation the technicians had a refresher training on malaria diagnosis methods by the Malaria Diagnostic Centre team, Centre Clinical Research, Kenya Medical Research Institute, Kisumu.

2.4.1 Quantitative buffy coat test

Approximately (55-65µL) of blood samples were filled into QBC capillary tubes by tilting the well-mixed blood tubes and placing the capillary tubes nearest to the blue lines in contact with the blood, keeping the tubes slightly above horizontal. The tubes were then rolled several times so as to mix the blood with the white anticoagulant coating, then tilted to allow blood to flow to the opposite end of the tubes into the orange reagent coating to mix with the acridine orange coating. The tubes were then sealed and a float inserted into each tube at the unsealed end and centrifuged at 12,000 rpm for 5 min. The centrifuged QBC tubes were then inserted into a paraviewer, two to three drops of fluorescence optical oil added for examination of the buffy coat area at 1,000× magnification using ParaLens Advance LED fluorescence attachment. The samples were processed and read at the study site by one of the study technicians.

2.4.1.1 Preparation of thick and thin blood films

Two thick and thin films per sample were prepared from each sample using6µL of blood to prepare thick film and 2µLfor thin film. The slides were air-dried and thin films were then fixed in methanol before the slides were singly stained with QBC F.A.S.T.™ or 10% Giemsa (Obare *et al.* 2013).

2.4.2 QBC F.A.S.T.™test

Thick films were individually flooded with F.A.S.T. Malaria stain for 10 min followed by fresh water for 5 min. Thin films were flooded with the stain for 45 sec and then rinsed by dipping in fresh water five times singly. Films were allowed to air dry vertically before examination. Examination of films was performed in a dark room with the aid of a ParaLens Advance LED fluorescence attachment at 1,000× magnification. A slide was

only considered negative if no parasites were detected after 100 fields were examined. Parasites were viewed as small fluorescent bodies with typical malaria morphological shapes.

2.4.3 Giemsa microscopy

Buffered water was used to prepare a 10% working Giemsa solution before staining. Blood films were individually flooded with the stain for 15 min followed by rinsing with water. Films were allowed to air dry vertically before examination. Examination of the films was performed with the aid of a light microscope at 1,000× magnification. A slide was considered negative if no parasites were detected on examining 100 microscopic fields.

All slides were read independently by two study technicians and a third reader (tie breaker) in case of discrepancy at the Malaria Diagnostic Centre, Centre Clinical Research, Kenya Medical Research Institute, Kisumu.

2.4.4 Quantification of parasite densities by QBC F.A.S.T.™ and Giemsa methods

Thick blood films were examined against 200 leukocytes. Parasite densities were estimated as parasites per volume assuming 8,000 leukocytes/µL of blood. If the parasites counted per microscopic field were 100 or more, then thin blood film examination was recorded by counting the number of parasitized cells against 2,000 red blood cells (RBCs) and converted to number of parasites per volume assuming 450,000 RBCs/µL of blood.

2.5 SD Bioline™malaria rapid test

Blood samples were added to the round wells followed by four drops of assay diluents into the square assay well. Results were read after 15 min. A negative result was indicated with the presence of one colour band, a positive result with two colour bands and an invalid result if the control line failed to appear and the test repeated with a new device.

A negative result by QBC, QBC F.A.S.T.™, SD Bioline™, and Giemsa microscopy was considered as true-negative because the risk for false positive microscopy results was considered low since the slides were all read independently by two experienced technicians and a third tie breaker.

2.6 Malaria parasite identification by PCR

Giemsa-stained blood films were soaked with 10 µL of phosphate buffered saline (PBS) 0.02 M, pH 7.4) then scraped off the glass slide by making circular movements with a sterile scalpel (Farla Medicals, Antwerp, Belgium). For each blood film, a separate scalpel was used. The collected material was transferred in a sterile 1.5-mL tube which contained 90 µL PBS. DNA was extracted with the QIAamp DNA Blood mini kit (Qiagen Benelux, Venlo, TheNetherlands) according to manufacturer's instructions. The standard used in the assay was the WHO International Standard for *P. falciparum* DNA Nucleic Acid Tests (NAT) obtained from National Institute for Biological Standards and Control (NIBSC; Hertfordshire, UK) as described by (Kamau *et al.* 2013).

2.6.1 Real-time PCR on blood films

Primers and probes for the amplification of the *Plasmodium* species were used to target PLU3 gene of all *Plasmodium* species and RNAse P, a human housekeeping gene, as described previously (Kamau *et al.* 2013). The assay was performed with the Applied Biosystems 7500 Fast Real-time PCR System, v.2.0.5 software. The thermal profile used for the qPCR was as follows: 5 min at 96°C, 40 cycles of 10secat 96°C; 30 sec at 60°C, with fluorescence collected at 60°C step. Each reaction contained 1 µL of template DNA and a reaction master mix containing Quantifast Probe Master Mix with Rox dye (QIAGEN,USA),10µMof each primer,5 µM of each probe, and dH_2O. All assays were run with the appropriate controls including non-template control.

Real-time PCR was performed on 40% of the discrepant results at the Malaria Drug Resistance Laboratories, Kenya Medical Research Institute, Kisumu Kenya. All laboratory personnel were blinded to the results from each of the tests.

2.7 Data Analysis

Data analysis was conducted using SPSS software package Version 20.0 (IBM SPSS Inc, Chicago, IL, USA) and GraphPadSoftware, Version 5 (GraphPad Software, Inc, La Jolla, CA, USA). The performance of the tests (QBC, QBC F.A.S.T.™ and SD Bioline™) against Giemsa microscopy was expressed as true-positive (TP), true-negative (TN), false-positive (FP), or false-negative (FN). The formulae used to calculate performance were TP/TP+FN for sensitivity (SS), TN/TN+FP for specificity (SP), TP/TP+FP for positive predictive values (PPV) and TN/TN+FN for negative predictive values(NPV). The results were interpreted with 95% confidence intervals (CI_{95}). Agreement between tests was determined by calculating Kappa statistics with 95% CIs and interpreted with the Landis and Koch classification. Relationship in parasite densities between Giemsa microscopy slide reads and QBC F.A.S.T.™ slide reads was determined using Pearson's correlation co-efficient. P values <0.05 were considered statistically significant.

3. Results

3.1 Performances of the tests

A total of 385 samples were tested. The total number of positive cases was found to be high with QBC test (310/385) as compared to the other tests (Table 1). Overal, SD Bioline™ had the highest sensitivity (91%) with QBC F.A.S.T.™ demonstrating a lower sensitivity (67%) when compared to the other tests. On the other hand, QBC test reported the lowest specificity of 30% as compared to 83% and 67% of QBC F.A.S.T.™ and SD Bioline™ tests respectively(Table 2). A total of 32 samples were found to be negative by both QBC, QBC F.A.S.T.™, SD Bioline™, and Giemsa microscopy.

Table 1. The total number of positives and negatives for each test in the study

Tests	Positive (%)	Negative (%)	Total
QBC	310 (81%)	75 (19%)	385
QBC F.A.S.T.™	185 (48%)	200 (52%)	385
SD Bioline™	242 (63%)	143 (37%)	385
Giemsa	198 (51%)	187 (49%)	385

QBC test gave the highest number of positive cases 310 (81%) while QBC F.A.S.T.™ 185 (48%) test gave the lowest number of positive cases as compared to the other tests.

Table 2. Diagnostic Performance of QBC, QBC F.A.S.T.™ and SD Bioline ™ using Giemsa as the gold standard and pair-wise comparison of concordant tests.

Tests	Sensitivity	Specificity	Positive Predictive Value(PPV)	Negative Predictive Value(NPV)	Kappa (95%CI)
QBC	90 (85-94)	30 (24-37)	58 (52-63)	74 (63-84)	0.45 (0.38- 0.52)
QBC F.A.S.T.™	77 (71-83)	83 (77-88)	74 (63-80)	78 (71-83)	0.61 (0.53-0.69)
SD Bioline™	91 (86-94)	67 (60-73)	83 (77-88)	87 (81-92)	0.58 (0.50-0.663)

SD Bioline™ had the highest sensitivity 91% followed by QBC and lastly QBC F.A.S.T.™ while QBC had the lowest specificity 30% as compared to the other tests

Three-hundred and eighty-five samples were tested by all the four tests, of which QBC and Giemsa microscopy had the highest number of discrepant results. One-hundred and thirty-one samples were found positive by QBC and not by Giemsa, hence 40% (*n*=52) of the discrepant results were randomly picked and further analysed by qPCR (Kamau *et al.* 2013) to ascertain the presence of *Plasmodium* DNA. Out of the 52 samples found to be malaria positive by QBC, only six samples (12%) had *Plasmodium* DNA by qPCR.

Assessment of agreement between tests using Giemsa as the gold standard showed a moderate agreement with QBC test (k=0.45) and SD Bioline™ (k=0.58) and a substantial agreement with QBC F.A.S.T.™ (k=0.61) (Table 2). The other tests had a rather poor agreement when compared to each other: QBC *vs* QBC F.A.S.T.™ (k=0.29), QBC *vs* SD Bioline™ (k=0.14) and SD Bioline™ *vs* QBC F.A.S.T.™(k=0.46) as shown in Table 3. The concordant rates of the tests relative to Giemsa microscopy were 90.4% (170/198; QBC), 77.3% (153/198; QBC F.A.S.T.™) and 90.9% with SD Bioline™ test.

Table 3. Levels of agreement for all the tests QBC, QBC F.A.S.T.™ and SD Bioline™ compared to each other

Tests	QBC VsQBC F.A.S.T.	QBC VsSD Bioline	SD Bioline VsQBC F.A.S.T.
Kappa values	0.29(0.20-0.39)	0.14(0.04-0.23)	0.46(0.37-0.54)

When the test agreement was compared against each they performed poorly with Kappa values of as low as 0.14.

3.2 Assessment of parasite densities from QBC F.A.S.T.™test compared to Giemsa

The continued implementation of different malaria control interventions aim at significantly reducing the morbidity and mortality associated with malaria, and possibly eliminate malaria. The move towards elimination will require more sensitive tests to match the expected reduction in parasitaemia (The malERA Consultative Group 2011). Consequently, we assessed the performance of the QBC F.A.S.T.™ test and Giemsa microscopy at different parasite densities (Fig.1). There were 153positive cases by both QBC F.A.S.T.™test and Giemsa microscopy. The positive cases were divided into three percentiles representing low parasitaemia (0^{th} and 25^{th} percentiles, *n*=37), moderate parasitaemia (26^{th} to 75^{th}percentile, *n*=77), and high parasitaemia (76^{th} to 100^{th} percentile, *n*=39) so as to determine how the two tests compared at different parasite densities. Overal, the two tests correlated relatively well when all the samples (*n*=153) were compared (r=0.645, *P*<0.0001; Fig.1A). At low parasite densities (0^{th} and 25^{th} percentiles) this relationship was maintained, albeit at a weaker level (r=0.361, *P*=0.028; Fig.1B). Similarly, at the moderate parasite density the two tests were significantly

correlated at a higher level than for the low parasitaemia (r=0.478, *P*<0.0001; Fig.1C). In addition, at high parasite densities (76th to 100th percentile) this relationship was still maintained (r=0.470, *P*=0.003; Fig.1D).

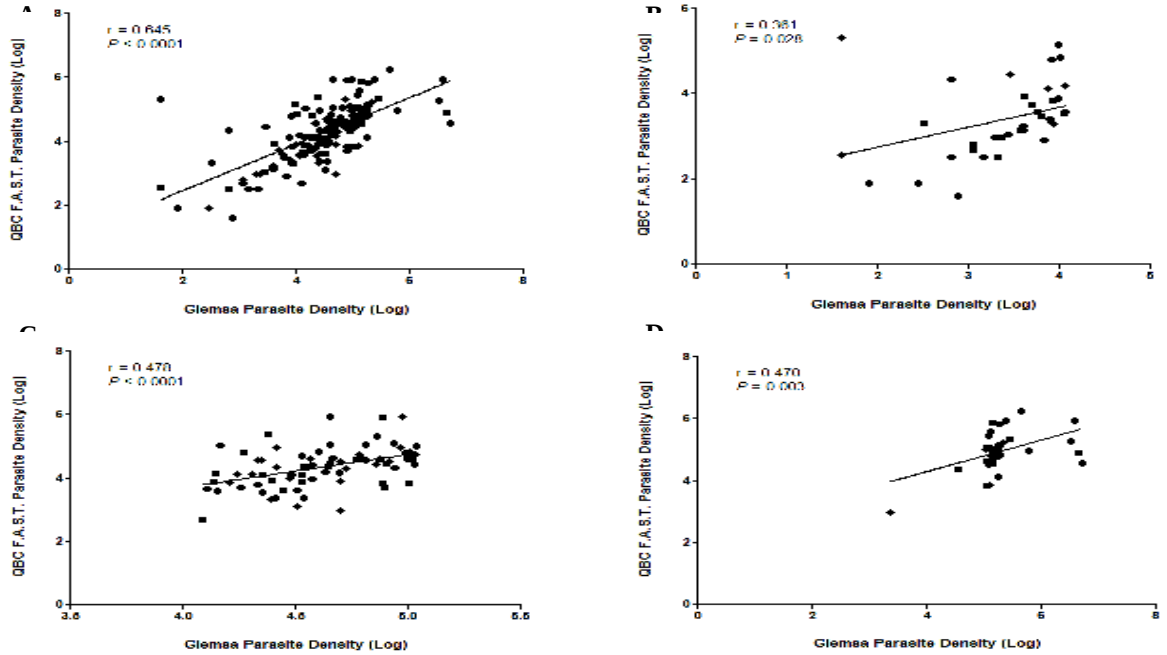

Fig.1 Relationship of parasite densities between QBC F.A.S.T. ™ and Giemsa tests
A) All positive samples (*n*=153); **B**) 0th to 25thpercentile (*n*=39); **C**) 26th to 75thpercentile (*n*=77); **D**) 76th to 100thpercentile (*n*=37). Analysis was performed using Pearson's correlation coefficient.
QBC F.A.S.T. ™ and Giemsa tests correlated relatively well (r=0.645, *P*<0.0001) on all positive cases (*n*=153) albeit they had a weaker correlation (r=0.361, *P*=0.028) at low parasitaemia (*n*=39).

3.3 Species identification by QBC F.A.S.T™ test compared to Giemsa

Of the 153 positive cases, QBC F.A.S.T.™ test detected 150 (98%)*P.falciparum*, zero (0%)*Plasmodium ovale*, zero (0%)*Plasmodium malariae*, and three(2%) mixed infections. Giemsa microscopy detected 141 (92.2%)*P.falciparum*, one(0.65%)*P.malariae*, one(0.65%)*P.ovale*, and ten (6.5%) mixed infections. Despite the difference in identification of the mixed infections by both tests, the difference was comparable between the two.

4. Discussion

The QBC and the QBC F.A.S.T.™ tests are fluorescent-assisted microscopy (FAM)-based methods. QBC had 90% sensitivity, a finding that is consistent with previous studies (Schindler *et al.* 2001 Kuladeepa 2012 Sandhya 2012). The high sensitivity is possibly enhanced through concentration of parasitized erythrocytes and the large volume of blood collected for examination (Kuladeepa 2012). However, the test showed a low specificity of 30%, possibly due to high rates of false positives as confirmed by the qPCR results, whereas only 12% of the discrepant results had *Plasmodium* DNA. The low specificity reported in this study is consistent with previous studies (Schindler *et al.* 2001, Morassin *et al.* 2002). This could be explained by the presence of Howell-jolly bodies and artefacts (Bhandari *et al.* 2008 Sandhya 2012). Concerns of leakage and breakage of blood-filled QBC tubes in the centrifuge are some of the pitfalls associated with this diagnostic approach as experienced in this study and by other studies (Pinto *et al.* 2001, Salmani 2011). Despite the low specificity, QBC holds promise as a good alternative in malaria diagnosis due to its speed and sensitivity as reported by Datta and his team (Datta 2010).

SD Bioline™ had the highest sensitivity compared to the other tests. The high sensitivity of SD Bioline™ kits, which detect HRP-II antigen, gives confidence that most of the malaria cases in the study population were diagnosed in agreement with previous reports (Kosack *et al.* 2013). Sensitivity improves with parasitaemia, however two cases with parasitaemia>10,000parasites/µL were diagnosed as negative. The false negative results could possibly be explained by the pro-zone effect (Kosack *et al.* 2013). A relatively low specificity of 67% obtained may have been due to the persistency of HRP-II antigen in the blood for up to 56 days after treatment (Nyunt *et al.* 2013), or the ability of the RDT to detect low parasite densities (Bell, 2002)or possible deletions of HRP-II (Gamboa *et al.* 2010), although this is rare in Africa (Baker and Kyle DE, 2005). These pose serious diagnostic challenge in malaria-endemic regions resulting in misdiagnosis with poor treatment outcome. Despite

the test being unable to detect non-falciparum malaria, it targets the most lethal *Plasmodium* species (Strom *et al.* 2013), hence greater impact. QBC F.A.S.T.™ test was found to be easy to apply since it uses the same sample preparation procedure as Giemsa microscopy. Positive cases were easily identified for parasitaemia levels >500 parasites/µL correlating to evaluation of a test that used the same fluorescence microscopy principle (Sousa-Figueiredo *et al.* 2010). However, the test demonstrated low sensitivity when compared to the other tests and this could be due to lack of clearly defined ring stages of the parasites.

The parasite densities in all positive cases by both Giemsa and QBC F.A.S.T.™ strongly correlated, indicating a good performance of QBC F.A.S.T.™ on quantitative diagnosis. The weak correlation at low parasite densities could be attributed to the field of view by Giemsa microscopy, which is visually clear, enhancing distinct parasite morphology, making parasite counting easier compared to the challenges obtained from QBC F.A.S.T.™ test where the morphology of the parasite is not clearly visible.

Accurate identification of *Plasmodium* species is critical because the results employed assist in correct deployment of specific control intervention strategies (Obare *et al.* 2013). Prompt and correct case diagnosis leading to accurate epidemiological assessments and optimal case management remains a critical research agenda, especially in malaria-endemic areas (Sousa-Figueiredo *et al.* 2010). Reliable differentiation of malaria infections is imperative since *Plasmodium* species differ in their biology, clinical symptoms and treatment regimens (Barber *et al.* 2013). Importantly, QBC F.A.S.T.™ was found to differentiate between *Plasmodium* species making it applicable to regions where falciparum and non-falciparum malaria cases are common. This was possible through the fluorescing of the parasites with typical malaria morphological stages. The test however is not very satisfactory in the diagnoses of *P. malariae* due to the lack of a very clear visual distinction of the morphology of the parasite. With the increased implementation of various control measures, significant reductions in malaria transmission intensities have been reported in some regions (O'Meara WP 2010). The reduction in transmission intensities is expected to be accompanied by reduction in malaria parasite densities, which will require more sensitive diagnostics for better case management and possible elimination. Despite malaria being a major cause of paediatric morbidity and mortality in most sub-Saharan African countries (Schumacher and Spinelli 2012), diagnostic tools development has remained slow and there is need for redoubled effort in the development of highly sensitive and robust point of care malaria diagnostics.

Study limitations were performance of real-time PCR on only the discrepant results obtained between the tests, and the use of Giemsa blood-stained microscopy as the gold standard for the entire study samples compared to using real-time PCR which has a better parasite detection limit. However, this was done because the risk of getting false positives was considered low as the slides were read by trained technicians and supervised by the Malaria Diagnostics Centre, Kisumu, Kenya. Real-time PCR is a very useful gold standard in that it is highly sensitive, easily reproducible and can detect cases with low parasitaemia missed by other tests. Its limitation is that the test is very expensive and time- and labour-consuming, hence it is used only to confirm the accuracy of microscopy (Johnston *et al.* 2006).

5. Conclusions

QBC test still remains a sensitive, rapid and accurate optical test although it should be supplemented with Giemsa due to limitations in species determination and parasite quantification. However, QBC is less sensitive compared to RDTs but the QBC F.A.S.T.™ test holds promise in rapid malaria diagnosis as differentiation of ring stage morphology is addressed.

Abbreviations

CI_{95}:Confidence intervals**;** CCR:Centre for Clinical Research; DNA:Deoxyribonucleic acid**;** GM:Giemsa microscopy; HRP-II:Histidine Rich Protein II; IMCI:Integrated Management of Childhood Illness**;** NAT:Nucleic acid tests**;** NPV:Negative predictive value; PBS:Phosphate buffered saline; PCR:Polymerase chain reaction**;** p-LDH: *Plasmodium*lactate dehydrogenase enzyme; PPV:Positive predictive value**;** QBC:Quantitative buffy coat**;** QBC F.A.S.T. ™:QBC (Fluorescence and Staining Technologies) Test; qPCR:Real-time polymerase chain reaction**;** RBC:Red blood cell**;** RDT: Rapid diagnostictests;SD BiolineTM:Standard Diagnostics Bioline™ Malaria Antigen *Pf* Test; SSC:Scientific Steering Committee; SS:Sensitivity; SP:Specificity; WBC:White blood cell**;** WHO:World Health Organization.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AAW, OP, NAK, KHL, OJM, and OB participated in the design of the study. AAW, AOA and AMH participated in data collection. AAW, OP, NAK, KHL, OJM, and OB participated in data analysis. All the authors made input in the writing of the manuscript. All authors read and approved the final manuscript.

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