Validation of a Biomeme Smartphone-Based DNA Real-Time PCR Assay for Diagnosis of Human Malaria at the Point of Care

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Summary

INTRODUCTION

Malaria presents a diagnostic challenge in most developing countries due to their financial status. In the year 2017, there were approximately 219 million new cases of malaria with more than 435 000 deaths globally. Children(70%) under five years were the most affected. In some African countries where transmission rates were high, a large population was infected but remained asymptomatic. Such patients had developed sufficient immunity to protect them from malarial illness but not from the infection. In these countries, cost and ease of performances are major considerations. Whereas microscopy and other contemporary methods for malaria diagnosis are available, development of an accurate, sensitive and cost-effective rapid diagnostic tool would go a long way in alleviating those challenges.

OBJECTIVES

This study tested a portable real time PCR (smartphone-based real-time) assay method tool from Biomeme *Inc.* in order to assess its ability in providing better diagnostic capability compared to existing methods.

MATERIALS AND METHODOLOGY

From April 2016 to February 2018 a study was carried out in Western Kenya. Nyando River basin which covers an area of 3517 km2. In five administrative wards; Ahero, Kabura, Kabonyo, East Kano and Awasi. Two and above years old patients who presented with malaria symptoms including axillary temperature of 37.5$_0$C or history of fever 48 hours prior to the mentioned ward's health facilities were recruitment. Consent of the 315 respondents including children whose parents or legal guardians signing for them was mandatory. Urine test for the presence of chloroquine, quinine or sulphonamides to confirm they had not taken anti -malaria drugs within the previous month was done. The initial *parasitemia* had to be between 1,000 to <200,000 parasites per µL of blood. Exempted were patients showing signs of severe anemia and malaria; those with a hemoglobin level of <5 g/d; presence of other diseases that causes febrile conditions; presence of any other *Plasmodium* species other than *falciparum*; and patients with a history of adverse events against ACT and sulphonamide drugs.

DNA was extracted using the Biomeme sample preparation kit and compared to that of the Chelex method. A NanoDrop spectrophotometer was used to measure the concentration, purity

and turbidity of the DNA obtained using the two extraction methods. Limits of detection of the DNA obtained from Biomeme and Chelex were determined by amplification of two *Plasmodium falciparum* gene markers, MSP1 and 18S rRNA. Student's t-test was used to compare the means of the two methods and a Kappa statistic value used to determine the level of agreement. Viral screening was performed before the actual blood collection.

During all surveys, nurses collected ~500µl of blood onto slides as thick and thin smears for microscopy onto filter paper for polymerase chain reaction (PCR) and into a HemoCue for immediate testing of hemoglobin levels (densities). Filter papers were dried at ambient temperature in the field, shipped to KEMRI and stored in plastic bags at -20°C containing silicate as desiccant. The samples were spun (350g, 10 min) and the pellets containing packed red blood cells (RBC), and white blood cells (WBC) were frozen with an equal volume of cryopreservation solution (0.9% NaCl, 4.2% sorbitol and 28% glycerol) and transported in liquid nitrogen container to KEMRI.

RESULTS

The Biomeme sample preparation kit recorded the highest yields and concentrations compared to the Chelex method. Biomeme DNA quantification method gave scores as yield of (33.39mg±8.36) while Chelex had yield of (7.6mg±2.48). The 18S rRNA amplification detection results on Biomeme and Chelex amplicons respectively were as follows; sensitivity (97.44±0.71%, 94.44±0.53%), specificity (50±0.71, 60±0.53%) with Kappa value (0.473, 0.544) while for MSP1, the respective results were as; sensitivity (97.37±0.49%, 91.67±0.43%), specificity (67±0.49%, 60±0.43%) and Kappa value (0.6401, 0.4755) p=0.05.

Evaluating the limits of detection, molecular marker in both gels was of 100 base pairs lane 1 and 2. That was representing positive control (3D7) and negative control (free DNAse water) respectively. It was evident that the amplicons intensities of Biomeme were much higher than those of Chelex. The gene fragment sizes were 200 bp on both gels, while serial dilution did not affect the intensities significantly across each gel using the 18S RNA as the gene marker.

CONCLUSION

These results suggest that, Biomeme was a rapid and accurate diagnostic technique for malaria that can be used at point of care despite the turbidity ratio which showed it had some *chaoatrophic* salts. Concentrations and the yields of the DNA depend on the platform method used for the extraction.

RECOMMENDATIONS

Biomeme could be an alternative method for resource-strained environments. It is portable and highly connected via web portal system for the transfer of data from one point to another. DNA extracted for molecular biological research work should be pure, free from contamination and toxicity leading to minimal DNA fragmentation.

Keywords: Biomeme, Malaria-diagnosis, Smartphone-based, *Plasmodium Falciparum*, Point-of-Care, Resource-Strained.

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Introduction

In the year 2017, there were approximately 219 million new cases of malaria with more than 435 000 deaths worldwide [44]. Children under the age of five years were the most affected at the rate of 70% [45].

In some African countries where transmission rates were high, a phenomenon was noted whereby a large proportion of the population was infected but remained asymptomatic [26, 21]. Such patients had developed sufficient immunity to protect them from malarial illness but not from the infection [30]. In such situations, finding malaria parasites in a febrile patient does not necessarily mean that the illness is caused by the parasites [43]. Further investigation would be required to identify the cause of illness.

In many malaria-endemic countries, lack of resources was a major barrier to reliable and timely diagnosis. Prompt, sensitive, specific and accurate diagnostic methods for proper patient management is essential. These effective and practical diagnostic methods will be of great impact and reduce the number of cases that go undiagnosed [43].

Although microscopy was the gold standard for malaria diagnosis, it was relatively laborious when large quantities of samples were to be processed and required expertise approach. A detection limit of 20 parasites / μL method was not sufficiently sensitive. The low sensitivity of microscopic technique for malaria diagnostics had a considerable negative impact on malaria control [5].

Previous advances in technology had led to the development of rapid diagnostic tests (RTDs) [20] which were easier to use but challenges concerning sensitivity, specificity and quantifications still remained unresolved [27]. One powerful diagnostic technique for malaria was use of polymerase chain reaction (PCR). That technique could detect drug-resistant parasites, mixed infections and was amenable to automation in processing large numbers of samples [40]. It was a more sensitive method in the diagnosis of malaria than quantitative blood count (QBC), RDT assays or convectional microscopy [28].

However, some PCR diagnostic methods face a number of challenges such as;

a. Unreliable electricity supply
b. Lack of complex instruments
c. Limited well-developed laboratory infrastructure [47].

Labor-intensive was hazardous especially if *ethidium* bromide, a *carcinogenic* DNA intercalating agent was used as part of the detection process. Moreover, DNA gel reading necessitated use of ultraviolet rays, which was a health hazard.

PCR can be remedied by using quantitative real-time PCR (QRT PCR) whose advantages include potential use to follow-up patients for malaria re-infection and recurrence. The study validated an improved, rapid, simpler, portable, genus- specific, cost effective smartphone-based real-time PCR assay method based on the *P. falciparum* 18S rRNA gene that could be used at the point of care for accuracy, sensitivity and specificity. This technology from Biomeme Inc. was compared to conventional PCR methods for limits of detection using the same set of primer sequences [19].

Materials and Methodology

Study Site

The study was carried out in Nyando River basin, Western Kenya. The basin covers an area of 3517 km_2. Five administrative wards; Ahero, Kabura, Kabonyo, East Kano and Awasi were recruited in the study. The five wards in the sub-county had similar characteristics making the end results generalizable.

Malaria transmission in that area followed the rain pattern. The long rain and short rain occurred in March-June and October-November respectively. Due to proximity to Lake Victoria, the area had a high average relative humidity at 65% with average annual temperature and rainfall of 17.3$_0$C and 1000-1800mm respectively. Included households who had been residents for at least six months prior to the study had been picked randomly until the required number was attained.

Inclusion and Exclusion Criteria

The study participants were residents of age two and above years old willing to consent to take part. Consent for children was sought from the parents of legal guardians. All respondents were required not to have taken anti-malarial drugs within a period of one month. This was confirmed by urine test for presence of chloroquine, quinine or sulphonamides.

All patients presented with malaria symptoms including axillary temperature of 37.5$_0$C or history of fever 48 hours prior to recruitment were considered. The initial *parasitemia* had to be between 1,000 to <200,000 parasites per μL of blood. The following were exempted from the study;

1. Patients who had taken anti-malarial drugs within a period of 14-30 days
2. Those who had declined to consent to participate in the study.
3. Patients showing signs of severe anemia and malaria.
4. Those with a hemoglobin level of <5 g/d.
5. Presence of other diseases that causes febrile conditions.
6. Presence of any other *Plasmodium* species other than *falciparum*

7. Patients with a history of adverse events against ACT and sulphonamide drugs.

Sample size

The sample size was calculated using the single population formula $n = z_2pq/e_2$ whereby;

- n : sample size
- Z_2 : the *abscissa* of the normal curve at $1-\alpha = 0.95$.
- e : the desired precision,
- p : the estimated proportion requiring known *N*.
- q : $1-p$ [16].

The following assumptions were taken into consideration; 95% confidence internal, 5% margin of error and a prevalence of 29%. The resultant sample size was 315. That calculated sample size factored in non-response of 0.9%. To obtain the number of samples to be collected from each site to ensure the sampled wards were equally represented in the study 315 was divided by 5. That obtained 65 study respondents per ward.

Sampling Procedure

Viral screening was performed before the actual blood collection was done. During all surveys, nurses collected ~500µl of blood onto slides as thick and thin smears for microscopy, onto filter paper for polymerase chain reaction (PCR) and into a HemoCue for immediate testing of hemoglobin levels (densities).

Blood samples from 315 patients with positive Giemsa-stained TBS were collected by venepuncture using EDTA vacutainer tubes or citratetubes (Becton Dickinson).The samples were stored at -20°C and thawed at 4°C prior to testing.

Dried blood spots were prepared on 3 MM paper Whatman-FTA-cards (Whatman, Florham Park, NJ) from blood samples collected from the respondents who attended the malaria clinic in the study sites. Filter papers were dried at ambient temperature in the field, shipped to KEMRI and stored in plastic bags at -20°C containing silicate as desiccant.

The samples were spun (350g, 10 min) and the pellets containing packed red blood cells (RBC), and white blood cells (WBC) were frozen with an equal volume of cryopreservation solution (0.9% NaCl, 4.2% sorbitol and 28% glycerol) and transported in liquid nitrogen container to KEMRI.

Informed Consent and Ethical Issues

Approval to carry out the study was sought and obtained from Kenya Medical Research Institute's (KEMRI) Scientific and Ethics Review Unit (SERU) under protocol reference number KEMRI/ SERU/CBRD/183/3757. Informed consent form was administered prior to collection of a blood sample. The risk to participating for children under five was minimal, since it was limited to temporary discomfort associated with finger prick for blood collection. No respondent was forced to participate in the study.

Procedures, information and purpose of the study was explained to the participants. After agreeing to all the provisions in the consent form, they were filled by the clinician, signed by the respondent and an independent witness. Strict confidentiality was maintained and all personal identifiers were removed from the data during analysis.

Microscopic Examination

Screening for malaria was performed using microscopy by examining 500 fields of Giemsa-stained thick and thin blood smears. Thick and thin blood smears were prepared from each individual in triplicate from finger-prick blood samples. The malaria blood smears (TBSs) were prepared and read by two blinded, experienced microscopists according to published protocols. For quantification of malaria parasites in the thick film, 200 white blood cells (WBC) were examined while simultaneously counting the malaria parasites. If the ratio of parasites to WBC exceeded 2 in the thick TBSs, the parasite density was evaluated from the thin TBSs.

The number of infected RBCs per 2000 total RBCs was counted. The approximate level of *parasitemia* was calculated. When the patient's baseline *erythrocyte* count was not available, it was assumed that 1 µl of blood contains 5 x 106 *erythrocytes*. The parasite counts were estimated with scores ranging from + to + + + +, whereby;

- + indicated 4 to 40 parasites/µL
- + + indicated 41 to 400 parasites/µL
- + + + indicated 401 to 4000 parasites/µL
- + + + + indicated >4000 parasites/µL of blood.

Parasitemia level was defined as negative if blood films showed absence of parasites in 300 microscopic fields.

DNA Extraction

DNA was extracted from 25µL of blood with the Biomeme prep sample kit (*Biomeme Inc., Philadelphia, USA*), according to the manufacturer's guidelines. It was eluted with 500 µL of elution buffer (Biomeme).

The second method of extraction was Chelex and was carried out as described [42] In the Chelex method, we used the blood spotted directly onto filter paper as described above under "sampling procedure" section.

DNA Quantification

The extracted DNA from each method was also analyzed by spectrophotometer. One µL of DNA extracted from each sample was placed directly on the spectrophotometer (NanoDrop, 2000, Thermo Scientific) according to the manufacturer's instructions and measures at 230, 260 and 280 nm. The system software provided the DNA concentration in ng/µL and automatically calculated the absorption ratio 260/280 (A260/280) and 260/230 (A260/230). Those ratios were used to characterize the DNA purity and turbidity respectively.

DNA Amplification

Limits of detection were carried out using DNA extracts from the two extraction methods. Two genes were used for each platform thus;

- 18S RNA
- Merozoite Surface Protein1 (MSP1).

DNA extracts were serially diluted ten-fold. MSP1 amplifications were performed by a two-step nested PCR. The products of outer (0.1µL) PCR were used for nested PCR as DNA templates. The master mix of the reaction had the following components;

1. dNTPs (400µM)
2. primers (100nM)
3. 5 units Taq pol, PCR water and
4. 1x PCR buffer to a final volume of 30µL.

The *thermocycler* conditions for both outer and inner PCRs were as follows;

- initial denaturation at 94$_o$C (3 minutes)
- followed by 30 cycles of denaturation at 94$_o$C (25 seconds)
- annealing at 50$_o$C (35 seconds)
- extension at 68$_o$C (2 minutes and 30 second).

Final extension was performed at 72$_o$C (3 minutes) and then halted at 4$_o$C.

The 18S RNA amplification was performed in one phase with a reaction volume of 30 µL. Master mix had the following components;

- DNAse-free water
- 1 x PCR buffer
- primers (500nM)
- dNTPs (250nM).

Conventional PCR thermocyler conditions were as follows;

- initial denaturation at 94$_o$C (3 minutes)
- followed by 30 cycles of denaturation at 94$_o$C (1 minute)
- Annealing and extension at 55$_o$C and 72$_o$C respectively for 2 minutes each.

Final extension at 72$_o$C (10 min.) before halting at 4$_o$C. Each experiment included control tubes corresponding to a serial dilution of;

(i) Positive controls consisting of *P. falciparum* (3D7) genomic DNA

(ii) A negative control containing no target DNA. During the detection of PCR products, 20µL of the PCR product was electrophoresed in a 1.5% *agarose gel* containing 0.1 µg of *ethidium bromide* per mL, and the resolved bands were visualized under UV trans-illumination.

Data Analysis

One-way ANOVA test using SPSS statistics software version 22 (*IBM, NY, USA*) was performed to examine whether or not there was significant difference in the average quantification values between the extraction methods.

Comparison of sensitivity and specificity between 18S RNA and merozoite surface protein 1 (MSP1) in the detection of *Plasmodium spp.* in clinical samples was examined with Chi-square and Cohen's kappa coefficient tests using SPSS statistics software. Cohen's Kappa values (k) calculation were performed manually and the following guideline for degree of agreement were used:

- poor $k < 0.00$
- slight $0.00 \leq k \leq 0.20$
- fair $0.21 \leq k \leq 0.40$,
- moderate $0.41 \leq k \leq 0.60$
- substantial $0.61 \leq k \leq 0.80$
- and almost perfect $k > 0.80$

VisionWorks®LSImage Acquisition and AnalysisSoftware were used to analyze the genetic diversity amongst the *Plasmodium* parasites.

Results

Quantity and Quality of DNA

The two methods generated a pellet of DNA at the end of each extraction. However, the color of the precipitated DNA varied across methods. The pellet colors ranged from clear, light yellow, and yellow to light brown and dark brown. Chelex DNA extraction method produced a light yellow, yellow and dark brown whereas Biomeme sample prep kit method generated clear pellets for the same samples. DNA suspension in both methods showed low viscosity.

Comparing the four methods by spectrophotometry, samples extracted by Biomeme prep kit produced more DNA concentration than the Chelex (66.74ng/ µL and 42.93 ng/µL respectively) (***Table 1***). Likewise, according to t-test, the average A280/230 and A260/230 differences were statistically significant between the two methods ($p < 0.0001$). The A260/A280 ratios for Biomeme (BO) method and Chelex (CO) method were 1.97 and 2.86 respectively and 0.01 and 2.35 in methods BO and CO for their ratio as seen in ***Table 1*** below.

Table 1: *Concentrations, Purities, Turbidities and Yields of DNA Extracted by Two Protocols*

Sample		Con.	A_{260}	A_{280}	A_{260}/A_{280}	A_{260}/A_{230}	Yield
BO	Mean	66.74±16.73	0.25±0.23	0.12±0.13	1.97±0.48	0.01±0.01	33.36±8.4
CO	Mean	42.93±32.88	1.07±0.75	0.34±0.38	2.86±0.67	2.35±1.76	7.69±2.48

Key: ***BO*** - Biomeme, ***CO*** - Chelex, ***Con*** - Concentration

Table 2: *Levene-Type Tests for a Trend and Homogeneity in the Group Variances of Concentration, Purity, Turbidity and Yield.*

		Ltev	ttem						95% cid	
		F	p-val	t	df	2 tail	md	se	lower	upper
Concentration	x	0.54	0.22	4.03	76	0	23.81	5.91	12.04	35.57
	y			4.03	56.44	0	23.81	5.91	11.98	35.64
Purity	x	3.57	0.06	-6.74	76	0	-0.89	0.13	-1.15	-0.62
	y			-6.74	68.48	0	-0.89	0.13	-1.15	-0.62
Turbidity	x	29.3	0	-8.34	76	0	-2.34	0.28	-2.90	-1.78
	y			-8.34	38	0	-2.34	0.28	-2.92	-1.77
Yield	x	23.7	0	18.4	76	0	25.7	1.4	22.92	28.48
	y			18.4	46.64	0	25.7	1.4	22.89	28.51

Key: **X** - equal variances assumed; **Y** - are equal variance not assumed respectively;
Ltev - Levene's Test for Equality of Variance; ***ttem-t*** - test for Equality of mean; ***md*** - mean difference;
se - standard error; ***F***-F - statistic; ***t-t*** - test; ***df*** - degrees of freedom; ***p - val***-significance value;
2 -tail-probability about the likelihood that the results have and ***cid*** - Confident.

Interval of the Difference

The Levene's test for equal variances from ***Table 2*** behind showed that homogeneity and trends in the means from ***Table 1*** behind were significantly different (2-tailed p<0.001). Therefore, showing that concentration, purity, turbidity and the yield were not equal across the two DNA extraction platforms. Indeed, the results showed that the concentrations and the yields of the DNA were dependent on the platform method used for the extraction as shown in ***figure 1*** below. The Biomeme sample preparation kit recorded the highest yields and concentrations compared to the Chelex method.

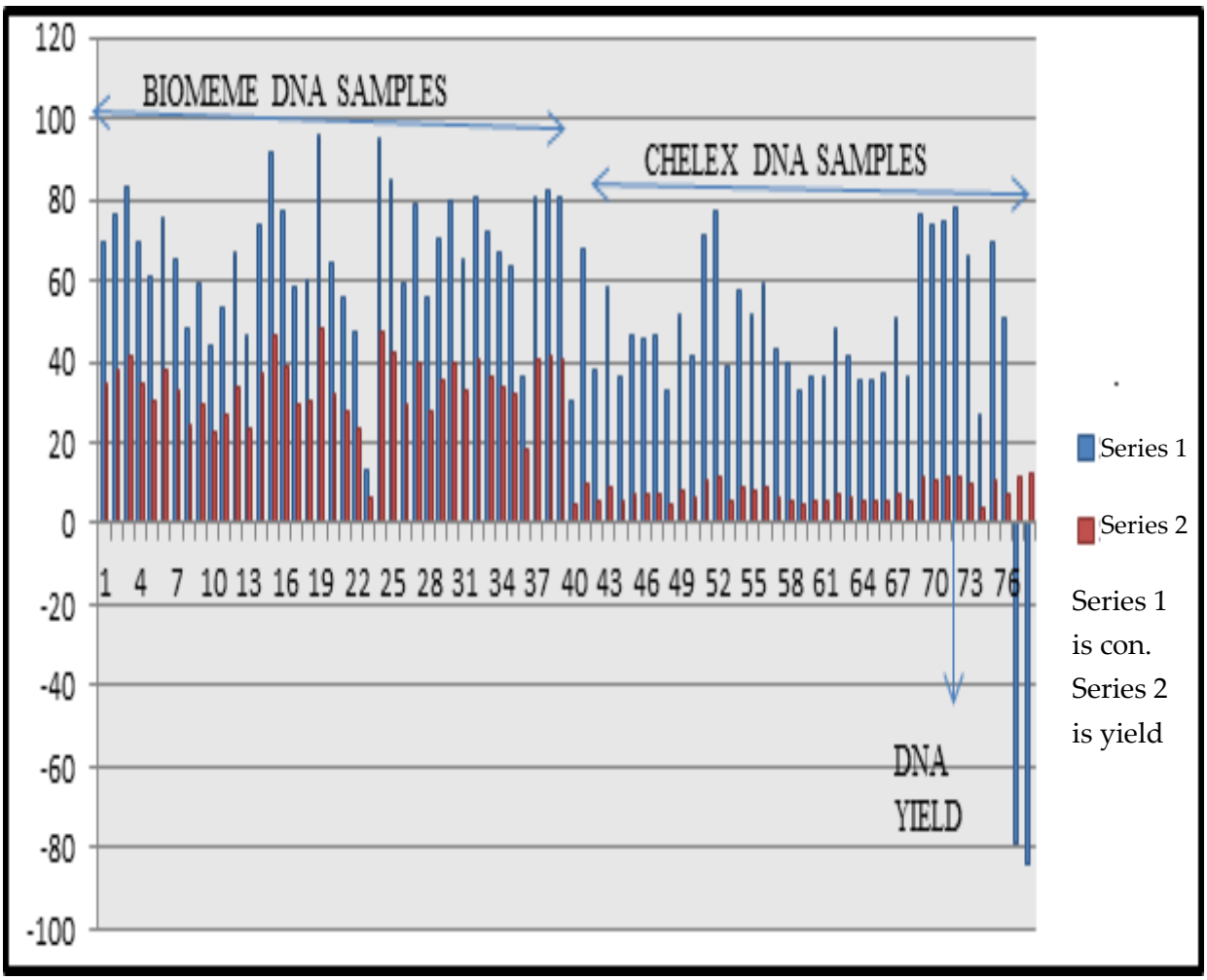

Figure1. *Comparative Combined Bar Graph of DNA Concentration and the Yield using the Two Extraction Platforms. The first 39 samples were from Biomeme samples prep kit and the 40th upto78th were from Chelex extraction method.*

Biomeme sample prep kit had superior DNA quality as compared to Chelex method ***(Figure 2***). ***Table 1*** pg.35 shows that Biomeme DNA extraction method turbidity range was low due to presence of *choatrophic* salts ions used within the Biomeme syringe column. The stronger absorbance at A230 may be an indicator of organic matter, phenolic ions and *thiocynate* ions. A good DNA range values should be 2.0-2.2.

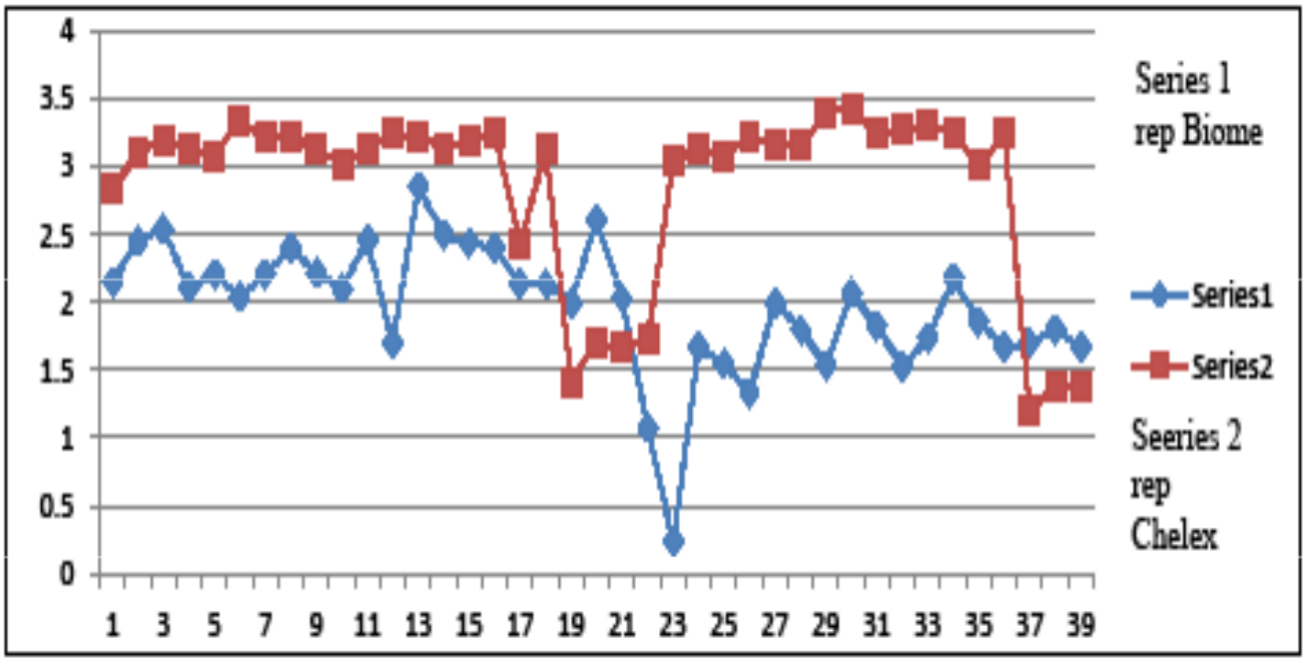

Fig 2.1 (a)

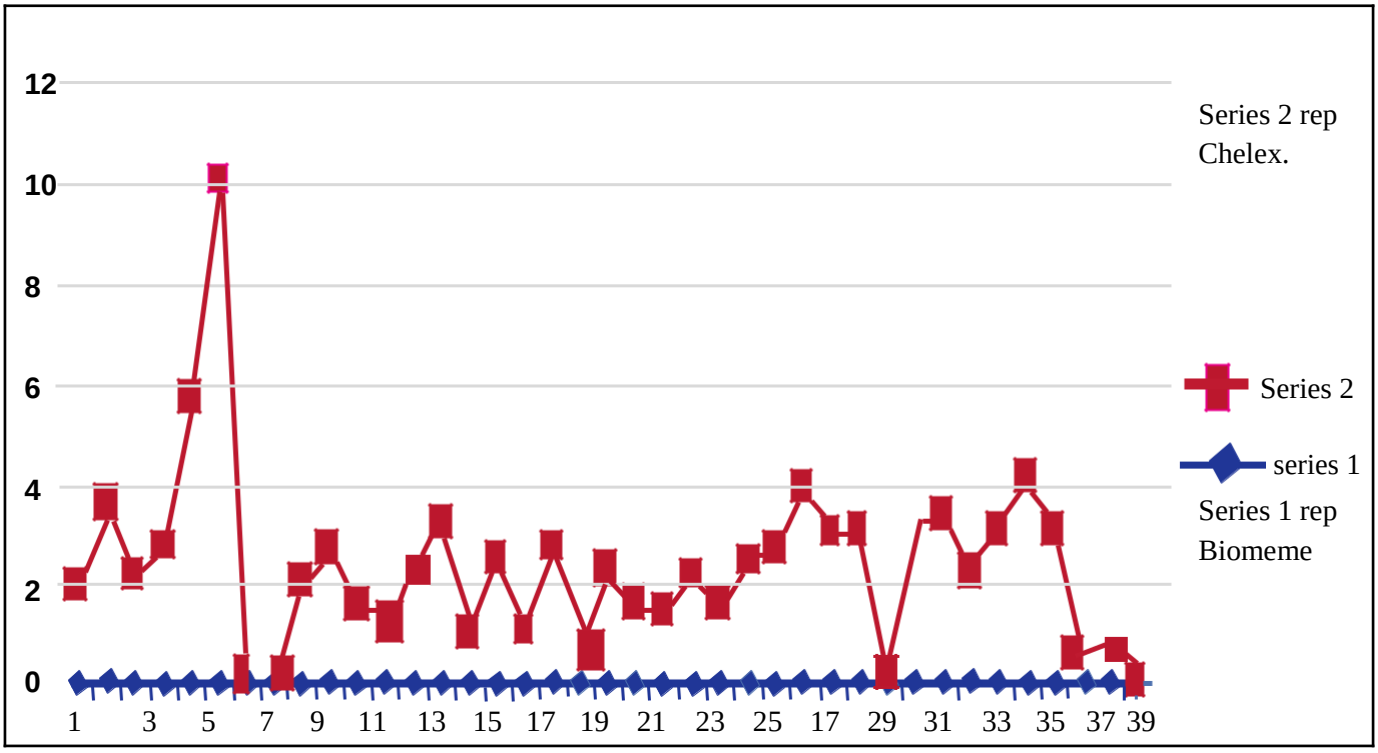

Fig 2.1 (b)

Figure 2: *The purity and turbidity comparative line graphs for the DNA extracts from the two platforms. The upper 2.1(a) and the lower (2.1.b) represent the purity and turbidity respectively.*

Evaluation of the Limits of Detection

The Biomeme DNA amplicons had a good precision in the respective ratios as compared to the Chelex (***Table 3***) ones. Thus promising as a reputable extraction platform for provision of molecular DNA. The respective Kappa values (k) and standard error of Kappa values SE (k) for the Biomeme (k)=0.473, SE (k)=0.363 and Chelex (k)=0.544, SE (k) =0.272 in ***Table 3(A)*** amplicons analysis were calculated at confidence interval of 95%. Therefore, it was indicative across the two DNA platforms that the levels of agreements were moderate ($0.41 \leq k \leq 0.60$). In ***Table 3*** (B), Biomeme had k=0.6401, SE (k)=0.2481 while Chelex k=0.4753 and SE (k)=0.2197. The degree of agreement were substantial ($0.61 \leq k \leq 0.80$) for Biomeme and moderate ($0.41 \leq k \leq 0.60$) for Chelex.

Table 3. The percentage of sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive value, negative predictive value, disease prevalence, combined sensitivity, combined specificity, true prevalence, and apparent prevalence of the Biomeme compared to Chelex amplicons using 18S RNA (***Table 3.A***) and MSP1 (***Table 3. B***) as the gene markers.

Table.3 set (A): *using 18S RNA* as the Gene Marker.

	SEN	SPE	PLR	NLR	PPV	NPV	DP	CS	CSP	TP	AP
Bio	97.44	50	194.9	5.12	97.44	50	95.12	99.80	80	92.70	95.12
Chel	94.44	60	236.1	9.26	94.44	60	87.80			82.90	87.80

Table .3 set (B): *using MSP1 as the Gene Marker*.

	SEN	SPE	PLR	NLR	PPV	NPV	DP	CS	CSP	TP	AP
Bio	67	67	292	3.94	97.3	66.7	92.68	99.78	86.8	90.24	92.68
Chel	91.67	60	229.2	8.33	94.4	50	87.8			80.49	85.37

Key for Table 3

set **A** and **B**:

SEN - Sensitivity **PLR** - Positive Likelihood Ratio **NLR**-Negative Likelihood Ratio **PPV** - Positive Predictive Value **CSP** - Combined Specificity **NPV** - Negative Predictive Value **DP** - Disease Prevalence **CS**- Combined Sensitivity **TP** - True prevalence **AP** - Apparent Prevalence. **SPE** - Specificity,

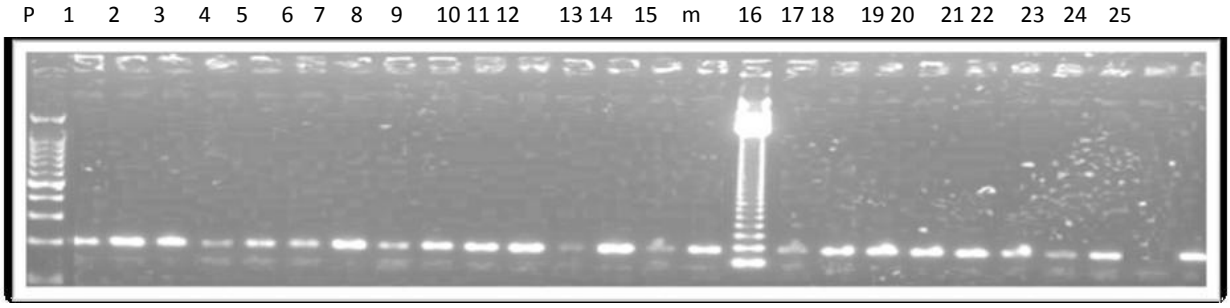

Figure 3 Panel A: *The gel results from Biomeme extraction*

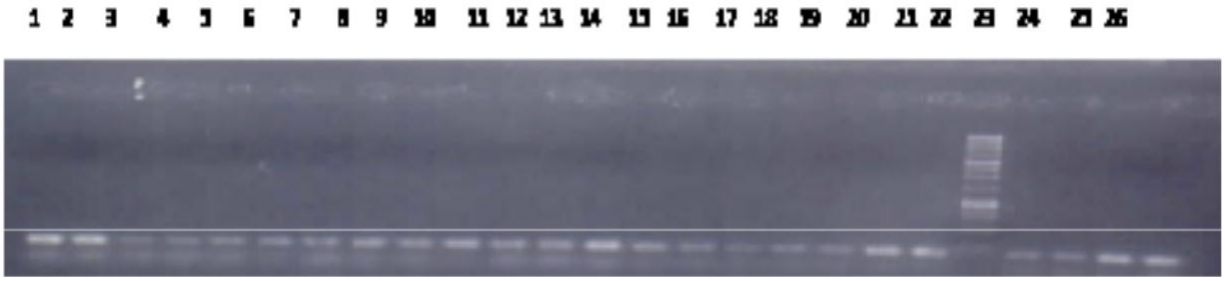

Figure.3 Panel B: *The gel results from Chelex extraction*

Figure 3. The gel results on *Fig.3 Panel A* are from Biomeme extraction while *Fig.3 Panel B* from Chelex extraction. The molecular marker in both gels were of 100 base pairs lane 1 and 2 on both gels representing positive control (3D7) and negative control (free DNAse water) respectively.

It was evident that the amplicons intensities of Biomeme were much higher than the Chelex ones (***Figure 3 panel B***). The gene fragments sizes were 200 bp on both gels. Serial dilution did not affect the intensities much across each gel using the 18S RNA as the gene marker.

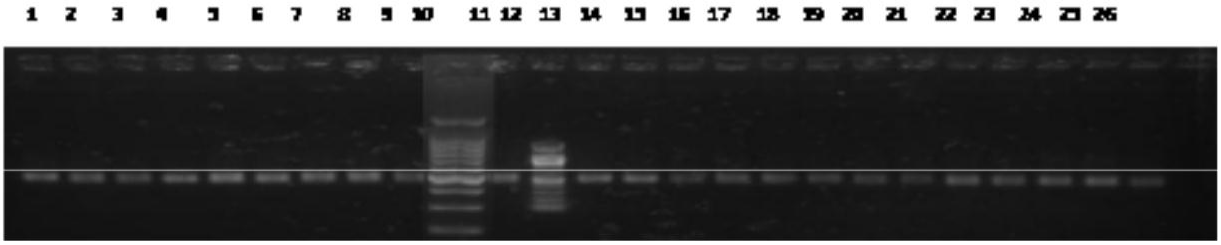

Figure 4 Panel n

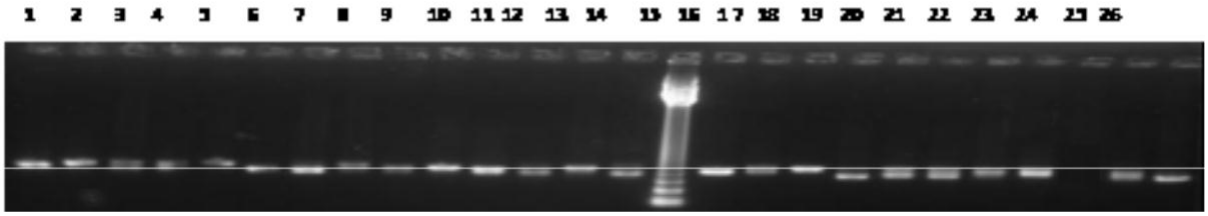

Figure 4 Panel m

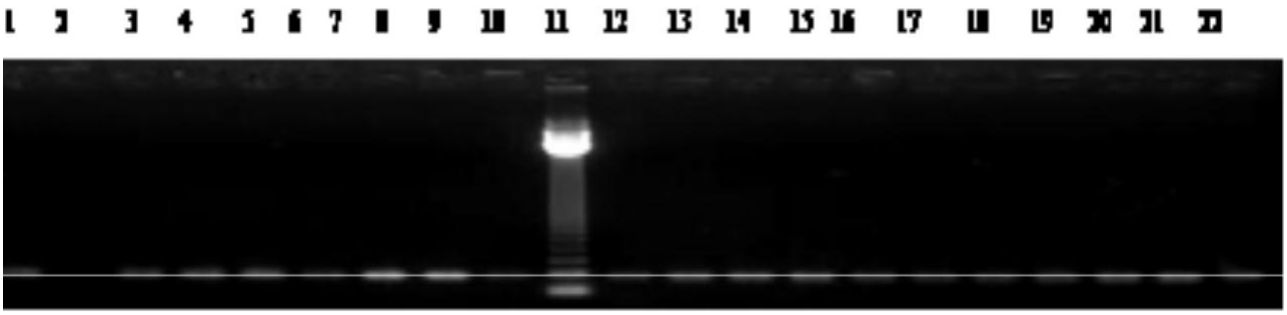

Figure 5 panel A: *from Biomeme amplicons*

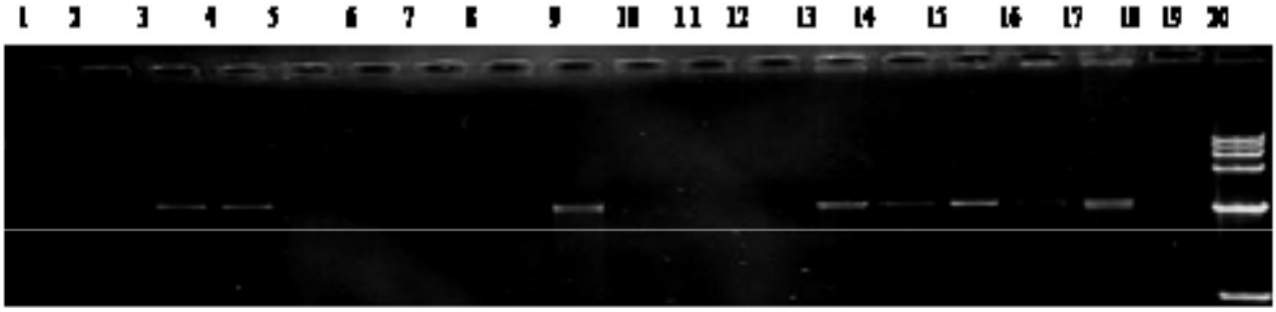

Figure 5 panel B: *from Chelex amplicons*

Figure 5: The gel on top row (panel A) was from Biomeme amplicons while the lower one (panel B) was from Chelex. All the respective DNA were serially diluted to 102 and amplified using 18S RNA as the gene marker. The gene fragment size was 200bp and the molecular marker (m) was of 100bp. Positive control was 3D7 and this was loaded in well 20 while the negative was loaded in well 19 on both gels. It was evident from it that, the intensity of the bands did change across the two DNA extracts from the two platforms. The latter ***(Fig.5 panel B)*** showed less sensitivity since some lanes did not show amplification.

Discussion

The application of DNA analyses in molecular science research depends largely on its purity, stability and genomic integrity [3]. Purity of DNA is important because impurities compromise accuracy, consistency and reproducibility of results. Having alternative approaches to purifying DNA from the cellular matrix could lead to improved sensitivity and accuracy of surveillance and diagnostic data which might promote timely treatment and the principal goal on eradication of malaria.

DNA extracted for molecular biological research work should be pure. Moreover, in order to be broadly applicable, the protocols for isolating DNA from the cellular matrix should be simple, affordable and produce DNA of good yield & quality. The assay method should also be rapid and reliable [3]. Other desirable qualities were that the assay method should be practical, free from contamination and toxicity and finally lead to minimal DNA fragmentation [6, 2, 46].

Some DNA extraction protocols, despite meeting a number of the above criteria, were undesirable because they utilize hazardous chemicals such as phenol, *chloroform*, *cetryltrimethyammonium* bromide and *isoamyl* alcohol [1, 39, 14, 32, 25]

Chelex method was reported to be simple, fast, effective and cheap. It involved less steps and also did not employ the organic solvents [42] hence, less hazardous to the user and the environment [13, 17]. When applied to a study of malaria parasites, the method had a sensitivity rate of 30 parasites/ µL. That was suitable for detection of low *parasitemia* in the field [35, 29]. However, it was labor-intensive and the purity of DNA from it was comparatively low in regard to the commercial kits [16].

Another demerit associated with the Chelex method was that, the DNA obtained was exposed to many cycles of preheating and thawing after storage [15].

Other commercial extraction kits had also been deployed in DNA extraction e.g. QIAamp DNA mini Kit that was widely used [38]. It was said to produce better results as compared to Chelex and boiling methods [12].

The other kit was Pure Rapid for Rapid Extraction / Procedure for Ultra Rapid Extraction (PURE) which was associated with Loop Mediated Isothermal Amplification (LAMP) [8]. GentraPuregene Blood Kit had shown a good DNA quality with a ratio of 1.8 for A260/A280 and that was an indication of a pure DNA [31]. Promega Wizard Genomic Purification Kit had been deployed as the standard for other protocols for extraction of *plasmodium* DNA [24].

From the recent study, Biomeme extraction platform had proved to be more efficient than Chelex. Biomeme extraction had higher DNA yield, concentrations and the DNA purity was better than the Chelex. However, the Chelex method had better turbidity ratio at absorbance A260/A230 as compared to Biomeme. The turbidity ratio of Biomeme could indicate that, it had some *chaoatrophic* salts which were not eliminated from the DNA. Overall, the Biomeme extraction kit was fast, cheap, less labor-intensive, user friendly, portable and little skill was needed as opposed to Chelex that was relatively labor-intensive, time consuming, expensive and required a higher degree of technical analytical knowledge.

Certain molecular methods had been developed for diagnosis or screening of malaria in asymptomatic individuals and deployed at point of care. Those included the PCR-NALFIA which used lateral flow as a read out. The sensitivity and the specificity ranges of that method were higher than those of Biomeme reported in this study [23]. However, the PCR-NALFIA method was costly. As a compromise, the Biomeme method might find wider applicability as a point-of-care method due to its cost effectiveness albeit a little less specific and sensitive.

Another method that had been used at the point-of-care was the RNA- hybridization method which was based on a *chemiluminescence* and had sensitivity rate of 100% and specificity was [11]. That method suffered the drawback of requiring expertise in its operation and was expensive to conduct. Semi-nested multiplex PCR was a simplified version of nested PCR and its sensitivity ranges from 94 to 98% and the specificity ranges from 98 to 100% [4]. The method finds limited utility at the point-of-care, required expertise in operation and was not cost effective, hence it was not broadly applicable in low-income settings.

PCR-Enzyme- Linked Immunosorbent Assay (PCR-ELISA) was a genus specific assay. It was quite rapid and produced less hazardous waste. Its sensitivity rate was 97% and the specificity is 96% [18]. The downside of that method was that, it was technically involving, expensive and not applicable as a point-of-care diagnostic method. The nucleic acid sequence – based amplification (NASBA) was an example of semi-quantitative isothermal amplification method with a fluorescence based read out. The method had a sensitivity of 94% specificity was 99% [23]. The disadvantages of that method include labor intensiveness and time consuming, hence it was not applicable as a point-of-care diagnostic assay.

In this study, we performed a comparative case study of conventional PCR and the nested PCR using DNA extract from two extraction methods (Biomeme sample prep kit and Chelex). From the results, it was clear that, both platforms had close sensitivities and specificities although the ones from Biomeme were relatively better than those of Chelex. Furthermore, it was clear that Biomeme amplifications had superior values compared to Chelex amplifications; this may be attributed to the superior quality and quantity of the DNA obtainable using the Biomeme platform. The low values of Chelex could be due to PCR inhibitors that were normally associated with that method [9, 33, 6]. Interestingly, nested PCR results obtained in this work were slightly better than those from the conventional PCR as opposed to early findings by [36]

Conclusion

Biomeme smartphone assay had proven to be a novel diagnostic platform, which incorporates accuracy, rapidity, sensitivity, specificity, reproducibility, robustness, cost-effectiveness, user friendliness and simplicity in its operation. Besides, it is portable and highly connected via web portal system for the transfer of data from one point to another. Areas suffering resource constrains are now easily and conveniently suited for this diagnostic method. Due to high cost of the existing PCR methods that limited their use in the

remote settings, we hereby highly recommend Biomeme smartphone assay to be implemented by the stakeholders in the health sector and Ministries of Health in regions afflicted by *P. falciparum* malaria.

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Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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