

Rapid Diagnostic Test Cassette: A Source of DNA for Malaria Diagnosis by PCR and a Quality Control Tool for the Test Performance

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Abstract

RDT is a malaria diagnostic tool used for early diagnosis of the disease. Though reliable, their challenging performance demands for continuous quality control monitoring. In this study, we evaluated the use of RDT cassette as a source of DNA to detect malaria parasite by PCR and in quality control of RDT in malaria diagnosis. Malaria parasite DNA was extracted from both immediately used ("fresh") and archived RDT cassettes and was amplified by PCR for *msp 1*, *msp 2* and *glurp* gene and the test performance of RDT was evaluated comparing PCR results for *msp 2* with those of microscopy and RDT. Findings from this study showed RDT as a source of malaria parasite DNA as DNA was successful extracted from RDT cassettes and amplified by PCR. Comparing PCR, microscopy and RDT results showed PCR to be most reliable in detecting malaria parasite. It was confirmed that RDT cassette is a potential cost effective source of DNA in molecular epidemiology studies to detect malaria parasite by PCR and in quality control of malaria diagnostic test performance.

Keywords: RDT, PCR, malaria parasite, DNA, microscopy, *msp 2*

1. Introduction

Malaria remains the most deadly tropical disease in the world, responsible for about 225 thousand clinical cases and 1 million deaths yearly. The greatest disease burden lies within the sub-Saharan region of Africa particularly affecting children and pregnant women (WHO, 2010).

In recent time, there is so much concern in the control of the disease among which are the use of Artemisinin based combination therapy (ACT), long lasting insecticide treated nets, Intermittent preventive treatments and case management of the disease (WHO, 2010). Case management defined by rapid diagnosis and prompt treatment is a control strategy of interest to minimize complications as well as reduce mortality. Rapid Diagnostic Test (RDT), which permits malaria diagnosis within 15-30 minutes, can be used at community level for case management (Tangpukdee *et al*, 2009; Chansuda *et al*, 2007; Moody, 2002). Though reliable, RDTs are limited in detecting sub-microscopic parasitaemia levels.

The World Health Organization (WHO) recommendations for performing quality assurance of RDTs among other things demands that quality control (QC) of RDT should be monitored in the field by comparing the results of RDTs with those of microscopy monthly (McMorrow *et al*, 2010; 2008; WHO, 2008; NVBCP, 2007). Though microscopy remains the goal standard for diagnosis, it

has been challenging using microscopy as reference tool for QC due to difficulties to re-read slides usually covered with fungi growth as a result of poor conservation facilities in local health centers. Alternatively, dried blood on geimsa stained slides could be collected and used as a source of DNA for PCR amplification to detect malaria parasite but the harsh staining of blood with geimsa may hamper the DNA extraction process and PCR amplification (Cnops *et al*, 2010; Scopel *et al*, 2004; Etoh *et al*, 1997)..

RDT is an immunochromatographic technique which contains fixed antibodies bound on nitrocellulose strips to detect soluble antigens of the parasite in blood to produce visible bands (Tangpukdee *et al*, 2009; Chansuda *et al*, 2007; Moody, 2002). Previous studies have shown RDT as a source of DNA for molecular analyses by PCR in the detection of malaria parasite (Cnops *et al*, 2011; Ishengoma *et al*, 2011, Veron and Carme, 2006). In this study, we investigated the use of Rapid Diagnostic Test cassettes as a source of DNA to detect malaria parasite by PCR and in quality control of RDT in malaria diagnosis.

2. Methods

2.1 Study Site

This study was conducted at the laboratory for Public Health Research Biotechnology (LAPHER-Biotech) at the Biotechnology Centre (Nkolbisson) of the University of Yaounde I, Yaounde, Cameroon.

2.2 Study Procedure

This study was part of the Research on the economics of ACTs (REACT) project in Cameroon. A malaria positive blood sample was obtained from the etoug-ebe Baptist Health Centre, Yaounde in 2010. About 5ul of the blood sample was dispensed into the sample well followed by 4 drops of assay diluents into the diluent well of 4 SD Bionline test kit for *P. falciparum* and pan malaria antigens according to the manufacturers' instructions. The RDT cassettes were allowed for 15-20 minutes and result were read. DNA was extracted from the 4 immediately used ("fresh") RDT cassettes and amplified by PCR for merozoite surface protein 1 (msp 1), merozoite surface protein (msp 2), and glutamate rich protein (glurp) genes of *Plasmodium falciparum*. DNA was then extracted from forty five 18 months old archived RDTs collected in 2009 and amplified by PCR for msp 2 gene to detect malaria parasite. As part of QC testing of RDTs, PCR results were compared with those of RDT and microscopy previously screened for malaria.

2.3 DNA extraction, PCR amplification and electrophoresis

The RDT cassettes (previously screened for malaria) were opened and the nitrocellulose membrane taken off. The portion containing the sample blotting side of the nitrocellulose membrane was carefully excised using sterile scissor and forcep and transferred in 1.5µl eppendorf tubes. DNA was extracted following the chelex boiling method as described by Plowe *et al*, (1995).

Amplification of msp 2, msp 1 and glurp genes was done by nested PCR using the T3 thermal cycler machine (Biometra) as described by Mbacham *et al*, (2008) using primers from Malaria Research and Reference Reagent Resource Center (MR4).

1.5% agarose gel (Seakem Nusieve) was prepared and was visualized under UV transilluminator (High Performance) and photographs taken using a digital camera.

2.4 Data and Statistical analysis

From the electrophoresis result, distance migrated by DNA was plotted against log of DNA

molecular weight marker to determine the molecular sizes of PCR products. The genetic diversity was calculated as the number of alleles present divided by the total number of distinct DNA fragments. Complexity of infection was defined as the average number of alleles present per individual was calculated as the total number of distinct DNA fragments divided by the total number of samples.

SPSS 16 statistical software was used for data analysis and graph plotting and confidence interval was taken at 95%.

3. Results

3.1 PCR, Microscopy and RDT

DNA was extracted from both "fresh" and archived RDT cassettes and successfully amplified by PCR (Figure 1). In total 41 out of 47 RDT samples were positive by PCR. Among these samples, 4 from "fresh" RDT cassettes were positive by PCR for *msp 2*, *msp 1* and *glurp* genes and 37 from archived RDT cassettes was positive for *msp 2* gene.

Comparing the PCR results with those of RDT and microscopy showed that all 4 "Fresh" RDT cassettes positive for malaria were all positive for malaria parasite by PCR. Among the 37 archived RDT samples positive by PCR, 12 were RDT and microscopy positive samples, 14 were RDT negative but microscopy positive samples and 11 were RDT positive but microscopy negative samples. From this results, PCR was more performant in malaria diagnosis as it could detect malaria parasite in both RDT and microscopy negative sample (RDT and microscopy false negatives). Results are shown on table 1.

3.2 Genetic diversity of *Plasmodium falciparum*

Genotyping the 37 *msp 2* PCR positive samples from archive RDT cassettes, genetic diversity of *P. falciparum* was 35.4% (17 alleles of 48 fragments) and complexity of infection was 1.3 (48 DNA fragments for 37 samples) (Table 2). The infection types were single or mixed with a dominance of single genotype in 28 samples (75.7%). Mixed infection was 24.3% (9 of 37) existing as 6 double (16.2%) and 3 triple (8.1%) mixed infections respectively (Figure 2). 17 alleles were detected with band sizes ranging from 400bp-630bp. The most dominant allele was the 500bp with a frequency of 38.3% (Figure 3).

4. Discussion

The introduction of RDT in malaria diagnosis has greatly improved on the case management of the disease and its control. Though this diagnostic tool is effective in early diagnosis of the disease, their test performance remains a major worry. This has edge WHO to recommend QC of RDT by monitoring their test performance using microscopy for at least 20 malaria positive and negative RDT samples (McMorrow *et al*, 2010; 2008; WHO, 2008; NVBCP, 2007). Microscopy remains the most effective tool for malaria diagnosis and used as the gold standard but their use in quality control remains challenging because it necessitates that control slides needs to be prepared alongside certain test RDTs (Igbiosa *et al*, 2010). Also, re-reads of the slides in a long run could produce false negative or positive results due to poor long term storage of the slides enhancing the development of fungi growth (Ishengoma *et al*, 2011).

Recently, RDT was shown to serve as a source of DNA to detect malaria parasite by PCR (Cnops *et al*, 2011; Ishengoma *et al*, 2011; Veron and Carne, 2006). In these studies, malaria parasite was detected in 18 months old archive RDT cassettes which were stored at room temperature. This suggests that PCR on "RDT" as a source of DNA could be used for QC to monitor the performance of RDT and will not be affected by the storage condition. From the results

obtained in this study, we confirm that DNA could successfully be extracted and amplified by PCR from both "fresh" and archived RDT cassettes. Comparing the results of PCR with RDT, and microscopy, PCR showed to be more reliable in detecting malaria parasite as PCR could detect malaria parasite in RDT and microscopy negative samples. Similar studies using RDT as a source of DNA showed PCR to be more effective than microscopy or RDT in malaria diagnosis (Cnops *et al*, 2011; Ishengoma *et al*. 2011; Veron and Carne, 2006). This confirms PCR on "RDT" as a more reliable tool than microscopy for QC in monitoring the performance of RDT in malaria diagnosis. Equally, in a study by Sandor *et al*, (2005), PCR on "RDT" showed similar result to PCR on filter paper as source of DNA when chelex method was used for DNA extraction suggesting that the DNA extracted from RDT cassette in this study was pure.

Merozoite surface protein 2 (msp 2) is a protein located on the surface membrane of malaria parasite (Kiwana, 2009). Due to the high polymorphic nature of the protein showing length polymorphism, it has been used in several studies to monitor the genetic diversity of malaria parasite (Ghanchi *et al*, 2010; Farnert and Bjorkman, 2005). As msp 2 was among the genes amplified to detect malaria parasite in this study, we also assessed the genetic diversity of msp 2 from the 37 PCR positive archived RDT samples. From our results, msp 2 showed limited diversity with a low complexity of infection. The alleles ranged from 400-630 bp and the 500bp was the most frequent.

The use of RDT as a source of malaria parasite DNA for molecular analysis by PCR could be of great importance to monitor the test performance of malaria diagnostic tools especially in QC monitoring of RDTs. Also, it could be used as an alternative to filter paper in molecular epidemiologic studies to monitor the genetic diversity, identify drug resistant markers etc. of the parasite. Most molecular epidemiology studies have relied on filter paper as a source of malaria parasite DNA which is costly. Thus, the use of RDT cassettes as a source of DNA will be a more cost effective source of DNA to serve for molecular epidemiologic studies especially in areas where limited studies on malaria are conducted.

5. Conclusion

This study confirms that newly used ("fresh") or archived RDT could be a potential source of DNA to detect malaria parasite by PCR and can serve in quality control monitoring of the test performance of RDT. The cost effectiveness of this source of DNA could be of great importance in molecular epidemiologic studies especially in areas of declining malaria prevalence where limited studies are conducted to enhance malaria control and elimination.

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Tables

Table 1: Amplification success of DNA extracted from archive RDT

RDT sample category	Number of RDT	PCR	
		Positive (%)	Negative (%)
	Fresh RDT		
RDT positive	4	4 (100%)	0 (0%)
	Archive RDT		
RDT and microscopy positive	12	12 (100%)	0 (0%)
RDT negative but microscopy positive	14	14 (100%)	0 (0%)
RDT positive but microscopy negative	11	11 (100%)	0 (0%)
RDT and microscopy negative	6	0 (0%)	6 (100%)
Total	47	41	6

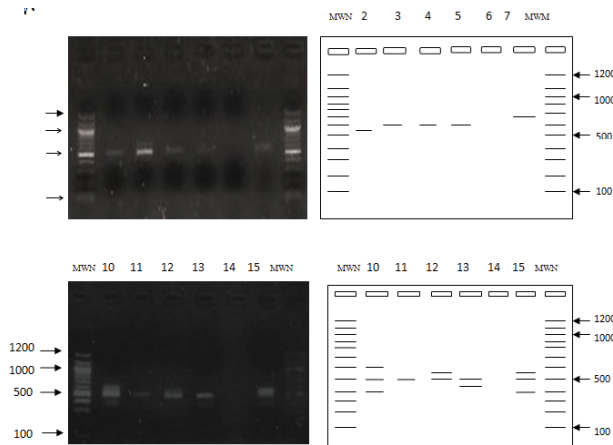
Table 2: Allelic distribution and complexity of infection for *Plasmodium falciparum*

Number of samples	Number of DNA fragments	Number of alleles	Genetic diversity (%)	Complexity of infection	Mean allelic size (bp)	Range of msp 2 alleles (bp)
37	48	17	35.4%	1.3	528.15 ± 57	400 - 620

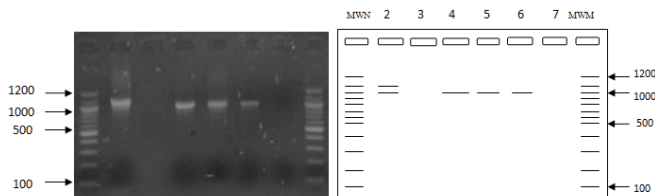
Legend: bp; base pair

Figures

A



B



C

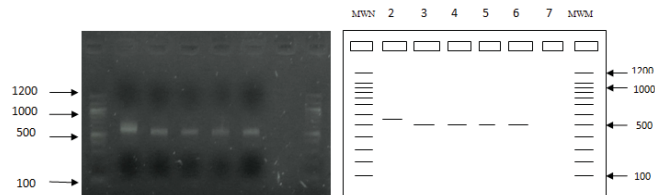


Figure 1: Agarose gel electrophoresis of nested PCR product

Legend: Lane MWN: Molecular weight marker (in base pairs), **3A:** Positive result for msp 2; lane 2-5 and 10-13, Negative result for msp 2; lane 6, Positive control; lane 7 and 15, **3B:** Positive result for glurp (4 RDT replicates); lane 4, 5 and 6, lane 2; positive control, lane 7; Negative result for glurp. **3C:** Positive samples for msp 1 (4 RDT replicates); lane 2, 4, 5 and 6, lane 1; positive control.

***P. falciparum* msp 2 infection**

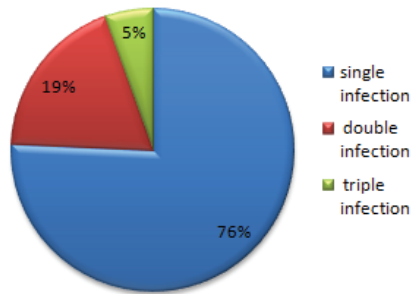


Figure 2: Distribution of *Plasmodium falciparum* msp 2 infections

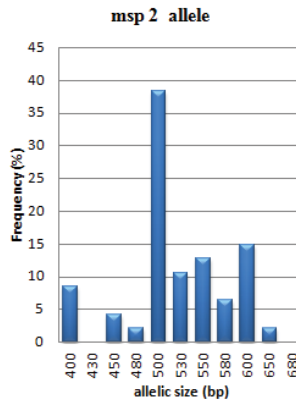


Figure 3: Allelic distribution of *Plasmodium falciparum* msp 2 gene

