

Evaluation of saliva-derived *Plasmodium falciparum* DNA using the OMNIgene®•ORAL kit in detection of malaria

Wilson Okumu¹, Winnie A. Okeyo¹, Elly O. Munde¹, Evans Raballah², Samwel B. Anyona¹, Collins Ouma¹ ¹ Maseno University, Maseno, Kenya

² Masinde Muliro University of Science and Technology, Kakamega, Kenya

Overview

In malaria holoendemic areas such as western Kenya, Plasmodium falciparum malaria is detected through microscopic examination of blood films from a suspected patient. The use of an invasive procedure to obtain blood as the primary source suffers from many challenges, which include: the pain associated with a needle that has turned away many would-be volunteers in clinical studies and the risk of inoculating the study participants with pathogenic microbes if proper sterilization is not performed at the point of injection. Therefore, an immediate need to develop a non-invasive, specific and sensitive method of detecting malaria and other diseases in routine health care services is desirable. DNA has been an important source of specimen in genetic variants and disease association studies.

Methodology

A cross-sectional study was conducted to evaluate the quality of saliva-derived DNA relative to traditional blood derived DNA of the same individuals with clinical symptoms of malaria (aged \geq 4 years; n= 100) and resident in *Plasmodium falciparum* holoendemic transmission region of western Kenya. Participants provided matched blood and saliva samples for microbial DNA using DNA Genotek's OMNIgene*•ORAL self-collection kit. The extracted DNA was subsequently used to genotype parasite Merozoite Surface Protein (MSP)-2. The MSP-2 parasite genotyping was carried out through nested PCR.

Background

- Globally, falciparum malaria accounts for the greatest degree of malaria-related morbidity and mortality (WHO, 2000).¹
- Traditional methods have always employed the use of Giemsa-stained thin or thick blood films in microscopy studies for detection of malaria parasitemia (Lieke *et al.*, 2004; Wongsrichanalai *et al.*, 2007).^{2, 3}
- Rapid diagnostic tests (RDT) or "dipstick", an antigen-based detection method, is used to detect protein markers of Plasmodium species in the blood of asymptomatic individuals. This approach still requires blood and therefore remains invasive.
- Taken together, these factors may reduce the rapid response rate in management of malaria, in the process posing a significant problem to malaria surveillance and control. Thus, an alternative source for malaria diagnosis that incorporates molecular tools is a necessity.

Purpose

To evaluate the quality of saliva-derived DNA relative to traditional blood derived DNA of the same individuals with clinical symptoms of malaria in western Kenya.

Study site and methodology

Study design: The study was carried out in a rural setting in western Kenya.

Individuals aged 4 years and above (n= 100) of both genders and presenting with clinical symptoms of malaria were recruited following written informed consent from the parents/guardians of the study participants.

DNA GENOTEK

For research use only, not for use in diagnostic procedures. Not available for clinical diagnostic use in the United States.

PD-WP-00035 Issue 2/2015-06 © 2015 DNA Genotek Inc., a subsidiary of OraSure Technologies, Inc., all rights reserved.

www.dnagenotek.com • support@dnagenotek.com

Ethical approval: The study was approved by the Maseno University Ethical Review Committee (MUERC).

Blood and saliva sample collection: Children presenting with clinical symptoms of malaria in a rural hospital setting in western Kenya were initially screened for parasitemia via Giemsa-stained slides and resolution on a microscope. All those that were positive for parasitemia provided additional 100 μL of blood, which was blotted onto FTA Classic cards (Whatman Inc., Clifton, NJ). Furthermore, additional saliva samples were collected using the OMNIgene•ORAL kit for collection of microbial DNA (DNA Genotek, Ottawa, ON).

DNA extraction: The saliva samples collected were extracted through the OMNIgene•ORAL protocols as per manufacturer's instructions (DNA Genotek, Ottawa, ON). Following complete drying of the FTA blood spots, DNA was extracted using the Puregene* DNA extraction kit Gentra* system (Gentra Systems, Inc., Minneapolis, MN) according to the manufacturer's instructions.

Quantification of DNA: DNA obtained from blood and saliva was quantified using NanoDrop 2000 UVVis spectrophotometer (Thermo Scientific, Waltham, MA).

Genotyping of parasite MSP-2 variants:

Plasmodium falciparum genotyping was performed on blood and saliva DNA extracts using nested PCR and MSP2 family-specific primers as previously described (Snounou *et al.*, 1999).⁴

Extraction protocol	Gentra	OMNIgene•ORAL
DNA source: Blood	40.2 ± 8.4 ng/µL	
DNA source: Saliva	_	56.3 ± 0.3 ng/μL

Table 1: Mean DNA concentration from saliva and blood The total DNA yield (from Gentra Systems and OMNIgene•ORAL/ saliva kit) was estimated by UV absorption based upon the mean A_{260}/A_{280} nm ratio. DNA yield from blood and saliva kit are comparable.

Some DNA Genotek products may not be available in all geographic regions.

*OMNIgene is a registered trademark of DNA Genotek Inc. All other brands and names contained herein are the property of their respective owners. All DNA Genotek protocols, white papers and application notes, are available in the support section of our website at www.dnaqenotek.com.

For research use only, not for use in diagnostic procedures. Not available for clinical diagnostic use in the United States.

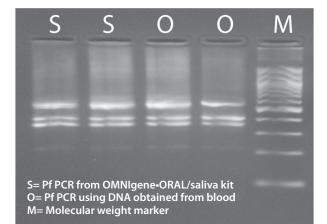


Figure 1: PCR results demonstrate DNA from the OMNIgene•ORAL/ saliva kits performs as well as DNA from blood in detection of circulating Plasmodium falciparum parasites.

Conclusions

- DNA from the OMNIgene•ORAL/saliva kits works the same as DNA from other sources (e.g., blood) in detection of circulating *Plasmodium falciparum* parasites.
- Future approaches should utilize saliva kits to avoid invasive blood sample collection for diagnosis and research.

Funding

- Pfizer Royal Society (Awarded to Profesor Collins Ouma for Best Scientist, 2010).
- OMNIgene•ORAL kits obtained from DNA Genotek Inc., Canada.

References

- ¹ WHO. 2000. World Malaria Report. WHO Press, Geneva Switzerland.
- ² Lieke *et al.*, Infect Immun.2004; 72: 6817-6824.
- ³ Wongsrichanalai *et al.*, Am J Trop Med. 2007; 77:119-127.
- ⁴ Snounou *et al.*, Mol Biochem Parasitol. 1993; 61: 315-320.