Saliva as a proven, non-invasive sample type for molecular malaria testing and surveillance using OMNIgene®•DISCOVER at ambient temperature

David Visanuvimol
DNA Genotek, Ottawa, Ontario, Canada
2014-09-19

Background

Saliva collection is a non-invasive alternative from RDT's for malaria detection and as a surveillance tool. Both *P. falciparum* and *P. vivax* DNA have been detected in saliva samples of malaria patients.[1, 2, 3, 4] It has been demonstrated that 1 mL of whole saliva samples harbours detectable levels of *Plasmodium* spp. DNA for downstream sequencing of *pfdhfr* and 18S rRNA.[5, 6] Therefore, saliva can be sampled for high sensitivity and specificity molecular-based malaria diagnosis. Additionally, participant compliance is increased with pain-free and easy saliva collection; therefore, providing greater access to *Plasmodium* DNA for improved monitoring of malaria transmission, identification of sub-patent or mixed *Plasmodium* species infections, and patient screening in artemisinin-resistance-emerging regions/elimination settings.

OMNIgene®•DISCOVER for sample stability and testing scalability

OMNIgene•DISCOVER enables non-invasive and pain-free collection of *Plasmodium* spp. via saliva. The OMNIgene•DISCOVER chemistry stabilizes *Plasmodium* DNA in 1 mL of saliva at ambient temperature for up to 1 year, eliminating the cost and complexity of cold storage making it ideal for field collection in remote and low-resource settings. The easy-to-use and reliable nature of the OMNIgene•DISCOVER kits improves patient compliance in both adults and children.

The following data is from ongoing pilot studies that show promising indications for the use of OMNIgene•DISCOVER as a non-invasive alternative for malaria diagnostics.

***P. falciparum* DNA from 1 mL of saliva is a reliable sample type for malaria detection (n=100)**
(Courtesy of Dr. Collins Ouma, Maseno University, Kenya, as presented at the 6th MIM Pan-African Malaria Conference in Durban, South African).

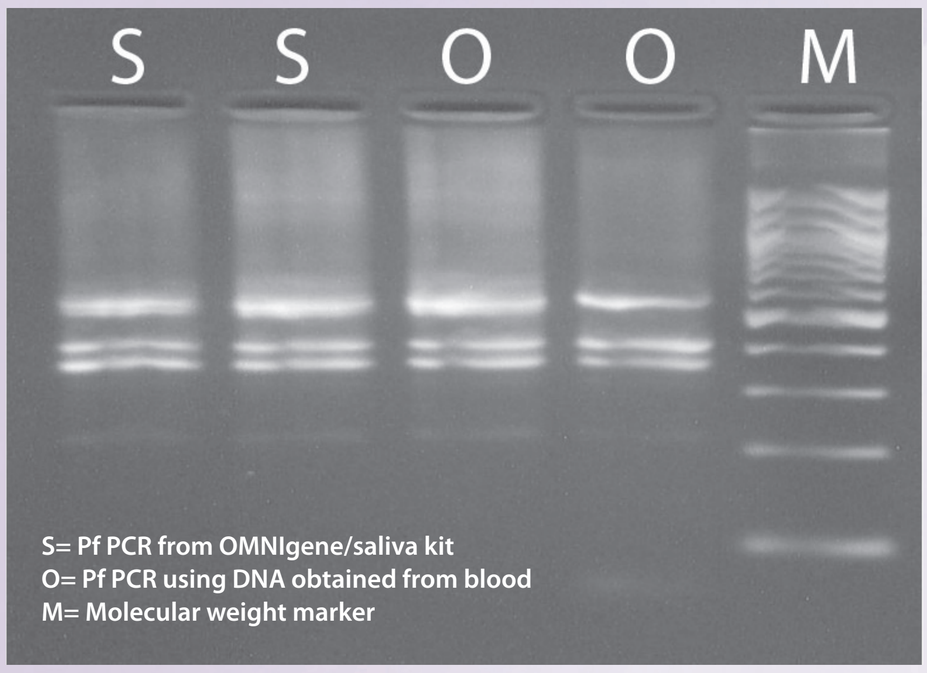
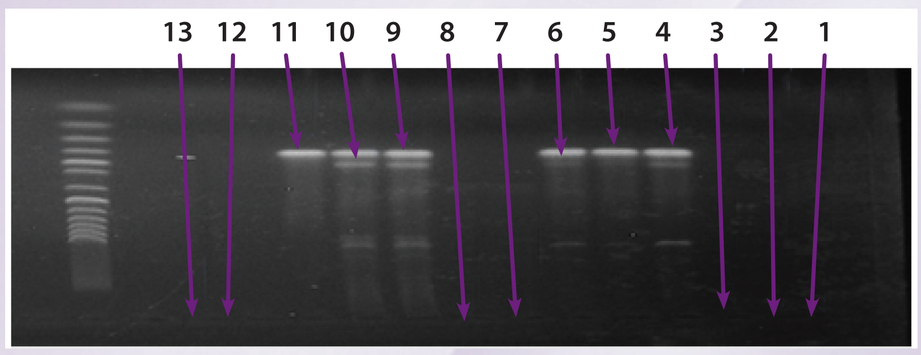


***Figure 1**: PCR results demonstrate DNA from the OMNIgene/saliva sample kits performs as well as DNA from blood in detection of circulating* Plasmodium *falciparum parasites.*

"I recommend that future approaches should utilize OMNIgene•DISCOVER self-collection kits to avoid invasive sample collections, improve patient recruitment, improve the patient experience, and enhance malaria diagnostics and research."

- Dr. Collins Ouma

1 mL of saliva stabilized in OMNIgene•DISCOVER can detect *P. falciparum* in symptomatic patients ranging from 6,000-70,000 parasites/µL (n=6)
(Courtesy of Dr. Deus Ishengoma, National Malaria Research Council, Tanzania).



Interpretations key

1. 21179 (saliva sample)
2. 21166 (saliva sample)
3. 21048 (saliva sample)
4. 21355 (saliva sample)
5. 21165 (saliva sample)
6. 21356 (saliva sample)
7. Negative control
8. Negative control
9. Positive *P. falciparum* control Pf (3D7)
10. Positive *P. falciparum* control (FCR3)
11. *P. falciparum* known sample
12. Positive *P. malariae* control
13. Negative control (ddH20)
14. 50 bp ladder marker

***Figure 2**: Agarose gel electrophoresis results of samples and control from PCR products.*

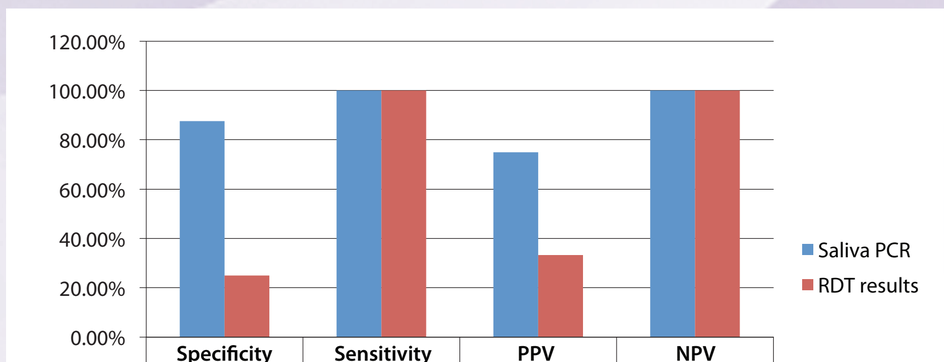
ID	Temperature (°C)	Microscopy	mRDT	PCR
21179	37.3	0/100	Negative	Negative
21166	38.3	Pf.313/200	Positive	Negative
21048	37.8	0/100	Negative	Negative
21355	39.0	Pf.1744/200	Positive	Positive
21165	37.6	Pf.158/200	Positive	Positive
21356	37.9	Pf.1064/200	Positive	Positive

***Table 1**: Patient information, microscopy, mRDT and PCR results.*

"The OMNIgene•DISCOVER kit proved successful in the majority of the tested samples with comparable results as detected by mRDT and microscopy. Therefore, the non-invasive OMNIgene•DISCOVER kit offers potential for use in malaria diagnosis."

- Dr. Deus Ishengoma

***P. falciparum* DNA collected from febrile participants shows high specificity and sensitivity compared to blood (n=11)**
(Courtesy of Dr. Kenji Obadiah Mfuh, John A. Burns School of Medicine, University of Hawaii at Manoa, USA)



	Specificity	Sensitivity	PPV	NPV
Saliva PCR	87.50%	100%	75%	100%
RDT results	25%	100%	33.30%	100%

"Malaria parasite DNA is present in the saliva of infected individuals and that saliva can be used as an alternative for non-invasive sample for the diagnosis of malaria in a PCR-based reaction. This pilot study also proves that the OMNIgene•DISCOVER kit is very effective in preserving malaria DNA at room temperature."

- Dr. Kenji Obadiah Mfuh

Additional testimony

"We have successfully extracted *P. falciparum* from the OM-501 kits. Parasite genetic diversity using MSP1 and MSP2 allelic families was also determined. I'm happy to say that we have sufficient amount of DNA for genetic analysis from the kits (n=69)."

- Dr. Magatte Ndiaye, Université Cheikh Anta Diop (University of Dakar), Senegal on the use of OM-501 for temporal dynamics of molecular markers of anti-malarial drug resistance in P. falciparum *parasite populations in Senegal.*

Conclusions

Given that only 14% of malaria cases are detected globally and several limitations exist to current diagnostic testing methods, there is a need to explore other approaches that enhance malaria diagnostics and national surveillance programs. OMNIgene•DISCOVER is a saliva-based collection and stabilization kit that allows for the non-invasive, proactive sampling and storage of *Plasmodium* DNA at ambient temperature.

References

1 Putaporntip et al. Improved performance with saliva and urine as alternative DNA sources for malaria diagnosis by mitochondrial DNA-based PCR assays. *Clin Microbiol Infect* 17: 1484–1491 (2011)
2 Nwakanma et al. Quantitative Detection of *Plasmodium falciparum* DNA in Saliva, Blood, and Urine. *J Infect Dis* 199:1567–74 (2009)
3 Gbotosho et al. Rapid Detection of Lactate Dehydrogenase and Genotyping of *Plasmodium falciparum* in Saliva of Children with Acute Uncomplicated Malaria. *Am J Trop Med Hyg* 83: 496–501 (2010)
4 Singh et al. Rapid detection of *Plasmodium vivax* in saliva and blood using loop mediated isothermal amplification (LAMP) assay. *J Infect* 67:245-247 (2013)
5 Ongagna-Yhombi et al. Improved assay to detect *Plasmodium falciparum* using an uninterrupted, semi-nested PCR and quantitative lateral flow analysis. *Malaria Journal* 12:74 (2013)
6 Singh et al. Comparison of three PCR-based assays for the non-invasive diagnosis of malaria: detection of *Plasmodium* parasites in blood and saliva. *Eur J Clin Microbiol Infect Dis* 33:1631–1639 (2014)

DNA genotek
Superior samples
Proven performance

www.dnagenotek.com • support@dnagenotek.com