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

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Background

The 2013 World Malaria report estimated there were 207 million malaria cases worldwide and an estimated 627,000 deaths in 2012.

On a global basis only 14% of malaria cases are detected.

Countries with high malaria burden reported lower case detection rates; 85% of estimated cases occur in countries where surveillance systems are weakest.

Limitations of current diagnostic testing

Current methods of identifying malaria infections are by antigen-based Rapid Diagnostic Tests (RDT's) and microscopy. These methods are labor-intensive, require supervision by healthcare workers and examination of thin/thick blood smears by skilled microscopists. Moreover, RDTs and microscopic examinations do not allow the detection of low-level, sub-patent malaria infections.

While RDT's have been instrumental in improving speed and precision of malaria diagnosis, the usage of blood and blood spots presents several barriers for RDT's, thereby impacting their adoption as surveillance tools. These barriers include:

- pain associated with finger pricking
- fear of contracting blood-borne diseases
- proper disposal of used needles
- physical damage and environmental susceptibility with RDT test strips
- need for supervision with RDTs to ensure correct interpretation of results and adherence to hygiene practices.

Moreover, while *Plasmodium* DNA can be extracted from dried blood blotted onto Whatman papers, poor extraction practice may result in cross-contamination and sub-standard quality and quantity of *Plasmodium* DNA. The obstacles associated with using RDT's and dried blood blots impact the implementation of these methods for nationwide malaria surveillance and monitoring programs.

Saliva as an alternative solution for malaria detection and surveillance

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•ORAL for sample stability and testing scalability

OMNIgene•ORAL enables non-invasive and pain-free collection of *Plasmodium* spp. via saliva. The OMNIgene•ORAL 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•ORAL kits improves patient compliance in both adults and children.

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Not available for clinical diagnostic use in the United States.

PD-WP-00041 Issue 3/2015-02

The utility of OMNIgene•ORAL kits in combination with highly sensitive PCR-based methods such as nested PCR and LAMP assays will facilitate the adoption of molecular-based malaria detection practices that will enable screening of samples from populations of all ages and genders, including high-risk groups such as pregnant women.

A saliva-based approach of *Plasmodium* DNA collection that ensures integrity of parasitic DNA regardless of humidity and temperature can effectively be scaled and integrated into national malaria detection and surveillance programs that require one centralized processing center and basic laboratory infrastructure.

The following data is from ongoing pilot studies that show promising indications for the use of OMNIgene•ORAL 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).

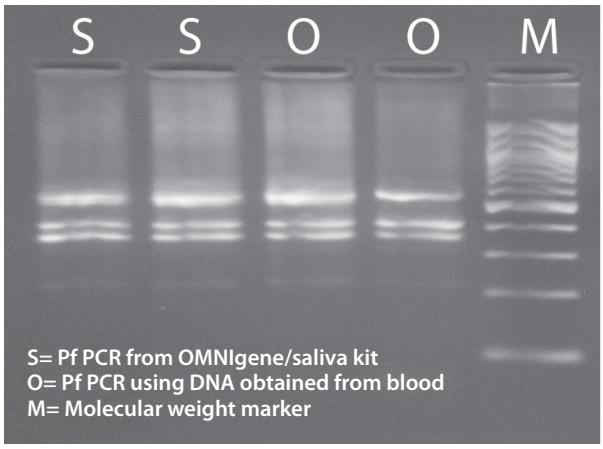


Figure 1*: PCR results demonstrate DNA from the OMNIgene/saliva sample kits performs as well as DNA from blood in detection of circulating* Plasmodium falciparum *parasites.*

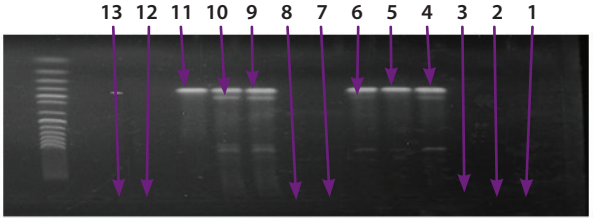
Extraction protocol	Gentra	OMNIgene
DNA source: Blood	40.2 ± 8.4 ng/µL	–
DNA source: Saliva	–	56.3 ± 0.3 ng/µL

Table 1*: Mean DNA concentration from saliva and blood. The total DNA yield (from Gentra Systems and OMNIgene/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.*

"I recommend that future approaches should utilize OMNIgene•ORAL 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•ORAL 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 Pmalariae control
13. Negative control (ddH20)
14. 50 bp ladder marker

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

ID	Temp. (OC)	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 2*: Patient information, microscopy , mRDT and PCR results.*

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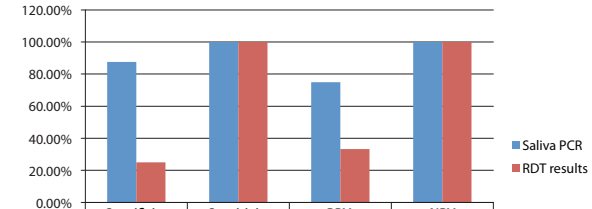
"The OMNIgene•ORAL kit proved successful in the majority of the tested samples with comparable results as detected by mRDT and microscopy. Therefore, the non-invasive OMNIgene•ORAL kit offers potential for use in malaria diagnosis."

- 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•ORAL 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•ORAL 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

PD-WP-00041 Issue 3/2015-02