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

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2014-09-19

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.
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).

**Table 1:** Mean DNA concentration from saliva and blood.

<table>
<thead>
<tr>
<th>Extraction protocol</th>
<th>Gentra</th>
<th>OMNIgene</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA source: Blood</td>
<td>40.2 ± 8.4 ng/µL</td>
<td>–</td>
</tr>
<tr>
<td>DNA source: Saliva</td>
<td>–</td>
<td>56.3 ± 0.3 ng/µL</td>
</tr>
</tbody>
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“*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).

**Table 2:** Patient information, microscopy, mRDT and PCR results.

<table>
<thead>
<tr>
<th>ID</th>
<th>Temp. (OC)</th>
<th>Microscopy</th>
<th>mRDT</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>21179</td>
<td>37.3</td>
<td>0/100</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>21166</td>
<td>38.3</td>
<td>Pf.313/200</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>21048</td>
<td>37.8</td>
<td>0/100</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>21355</td>
<td>39.0</td>
<td>Pf.1744/200</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>21165</td>
<td>37.6</td>
<td>Pf.158/200</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>21356</td>
<td>37.9</td>
<td>Pf.1064/200</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

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

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