CRISPR-based Ultrasensitive Diagnostics for Malaria

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Conflict of Interest Disclosure

- No financial relationships with a commercial entity producing healthcare-related products and/or services.
Global Burden of Malaria

In 2017 there were an estimated 219 million cases with 435,000 deaths.

Nearly Half the world population is at risk of malaria.

In 2007 the WHO endorsed the goal of Malaria Elimination and Eradication.
Children under 5 years are the most vulnerable group affected by malaria, accounting for 61% of all malaria deaths worldwide.

Every 2 minutes, a child dies of malaria.
In 2017 there were an estimated 276 million Rapid Diagnostic Tests (RDTs) sold globally.

Most RDTs (66%) detected *P. falciparum* only and were supplied to sub-Saharan Africa.

In sub-Saharan Africa, RDTs are becoming the most used method to test for malaria. In 2017, an estimated 75% of malaria tests were conducted using RDTs, up from 40% in 2010.
Most currently available Rapid Diagnostic Tests (RDTs) work by detecting *P. falciparum* histidine-rich protein II (HRP2) antigen

**2010** first confirmed identification of *P. falciparum* parasites with *pfhrp2/pfhrp3* gene deletions

- Significant increase in Peruvian Amazon from 20.7% during 1998-2001 to 40.6% during 2003-2005.
- Lower prevalence in other countries but HRP2 deletions found in Eritrea, Ghana, Kenya, Rwanda, and India
<table>
<thead>
<tr>
<th>Parasitemia %</th>
<th>Parasites per microliter</th>
<th>BinaxNOW Sensitivity: <em>Plasmodium falciparum</em></th>
<th>BinaxNOW Specificity: <em>Plasmodium falciparum</em></th>
<th>BinaxNOW Sensitivity: <em>Plasmodium vivax</em></th>
<th>BinaxNOW Specificity: <em>Plasmodium vivax</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Presume MCV 80fL (60fL)</td>
<td>&gt;5000</td>
<td>99.7% (326/327)</td>
<td>94.2% (3297/3500)</td>
<td>93.5% (462/494)</td>
<td>99.8% (2863/2870)</td>
</tr>
<tr>
<td>&gt;0.04% (0.03%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0.0008% - 0.04% (0.006% - 0.03%)</td>
<td>1000-5000</td>
<td>99.2% (126/127)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00008% - 0.008% (0.0003% - 0.006%)</td>
<td>100-1000</td>
<td>89.2 - 92.6% (33/37 - 25/27)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 0.00008% 0 – 0.0006%</td>
<td>0-100</td>
<td>53.9% (21/39)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
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<td>23.6 - 47.4% (34/144 - 37/78)</td>
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<tr>
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<td></td>
<td>6.2% (8/129)</td>
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</tbody>
</table>
Ultrasensitive Detection Needed for Malaria Eradication

Prevalence data with 95% CI from 86 surveys containing both adults and children, and fitted model (blue line) with 95% CI of the mean (light blue area)

Estimated average sensitivity of microscopy and 95% CI of the mean in all-age surveys according to underlying PCR prevalence

The following preferred product characteristics for new technologies were discussed at the meeting:

- An ability to detect parasitaemia of ≤2 parasites/μl.
- Need for a sample volume of not more than 50μl blood.
- An assay that is not instrument specific.
- Flexibility in power supply.
- An ability to detect malaria parasites at genus level and then conduct species differentiation on positive samples.
- Results should ideally be available within 16 hours (same working day or early on the following day for patients providing samples just before closing hours), with a maximum waiting time of 24 hours for results.
- The assay should allow processing of 48 samples/person/platform/day.
- Reagents should be stable at 4°C for a minimum of one year, and at room temperature for a minimum of six months.
So what’s needed for future malaria diagnostics?

<table>
<thead>
<tr>
<th>High Density Infections</th>
<th>Medium Density Infections</th>
<th>Low Density Infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Plasmodium falciparum</em></td>
<td><em>Plasmodium falciparum</em></td>
<td><em>Plasmodium falciparum</em></td>
</tr>
<tr>
<td>Non-falciparum malaria (<em>P. vivax, ovale, malariae, knowlesi</em>)</td>
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</tr>
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</table>
Field Evaluation of a High Throughput Loop Mediated Isothermal Amplification Test for the Detection of Asymptomatic *Plasmodium* Infections in Zanzibar

Berit Aydin-Schmidt¹⁺, Ulrika Morris¹, Xavier C. Ding³, Irina Jovel¹, Mwinyi I. Msellem⁴, Daniel Bergman¹, Atiquil Islam¹, Abdullah S. Ali¹, Spencer Polley⁵, Iveth J. Gonzalez⁵, Andreas Mårtensson⁶, Anders Björkman¹

Table 3. Diagnostic accuracy of Malaria pan HTP-LAMP compared to PCR for 3008 field samples.

<table>
<thead>
<tr>
<th></th>
<th>PCR +</th>
<th>PCR -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTP-LAMP +</td>
<td>20</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>HTP-LAMP -</td>
<td>29</td>
<td>2957</td>
<td>2986</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>2959</td>
<td>3008</td>
</tr>
</tbody>
</table>

p < 0.001*

- **Sensitivity**: 40.8% (95% CI 27.0–55.8%)
- **Specificity**: 99.9% (95% CI 99.8–100%)
- **Positive predictive value**: 90.9% (95% CI 70.8–98.9%)
- **Negative predictive value**: 99.0% (95% CI 98.6–99.3%)

* by McNemar’s test.

doi:10.1371/journal.pone.0169037.t003
**Target Plasmodium DNA present**

- Specific High Sensitivity Enzymatic Reporter UnLOCKing Cas12a Enzyme Plasmodium-specific guide RNA

**Target dsDNA**

- Non-target dsDNA

**Inactive Cas12a**

**Active Cas12a bound to target dsDNA**

**Quenched ssDNA Reporter**

**Fluorescent cleaved ssDNA Reporter**

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**Cas12a detection**

Collateral cleavage produces fluorescence

- No Target DNA present

Un-activated Cas12a results in no collateral cleavage of ssDNA reporter and releases no signal

- RPA

- Target dsDNA

- Non-target dsDNA

- Inactive Cas12a

- Active Cas12a bound to target dsDNA

- Quenched ssDNA Reporter

- Fluorescent cleaved ssDNA Reporter
Pan-plasmodium target
Falciparum specific target
Vivax specific target
40 p/μL  2 p/μL
0 aM  1 fM  50 aM
One-pot SHERLOCK reaction proceeds at 40°C for 60 minutes
Acknowledgements

- Wyss Institute
- James McGee
- Nico Angenent-Mari
- James Collins
- Nira Pollock
- Jeffrey Dvorin
- Helena de Puig Guixe
Questions/Comments?