PCR detection of Plasmodium species

Methods

Existing primers for *Plasmodium falciparum*, *P. malariae*, *P. ovale* and *P. vivax* 18s rRNA, and nested PCR conditions were taken from [1]. Template DNA was extracted from blood samples spotted onto Whatman® paper using a Chelex-100 isolation technique [2].

Results

All blood samples were infected with *P. falciparum*, no blood was infected with *P. ovale* or *P. vivax*, while two samples contained *P. malariae* in addition to *P. falciparum* (Table S3).

Table S3: Scoring of presence or absence of four species of *Plasmodium* parasite following PCR detection.

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>P. falciparum</em></th>
<th><em>P. malariae</em></th>
<th><em>P. ovale</em></th>
<th><em>P. vivax</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>SH158 (triple infection_#1)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SH93 (triple infection_#2)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SH79 (double infection_#1)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SH25 (double infection_#2)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K10 (single infection_#1)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IG14 (single infection_#2)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: +; Present, -; Absent

Smith et al. (2011)
Alternative splicing of the *Anopheles gambiae* Dscam gene in diverse *Plasmodium falciparum* infections

*Plasmodium falciparum* parasite intensity

**Methods**

Real-time quantitative PCR assays were performed on an Applied Biosystems StepOnePlus cycler and we used the non-specific nucleic acid stain SYBR Green I which preferentially binds to dsDNA. For our reference gene, human-specific Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers were designed using Primer3 software and checked for quality (tendency to dimerise, cross-dimerise, hairpin etc) with NetPrimer (primers were: F: 5’ – CGACCACTTTGTCAAGCTCA – 3’, R: 5’ - GGTGGTCCAGGGGTCTTACT – 3’) and amplified an 112bp fragment. Existing primers were used to amplify *Plasmodium falciparum* SSU rRNA (primers were: F: 5’ – TCTAGGGAACTATTTTAGCTT – 3’, R: 5’ – CACAGTAAATGCTTTAAGTT – 3’) isolating an 180bp fragment [3]. All cycler runs included serial dilutions of parasite DNA to indicate primer efficiency and included a melting curve analysis for product identification. Each reaction contained: 8µl SYBR Green Master Mix, 10µl ddH₂O, 1µl primer mix (10mM concentration) and 1µl template genomic DNA. PCR conditions were:- holding stage: 95ºC for 10 minutes; cycling stage: 40 cycles of 95ºC for 15 seconds, 55ºC for 60 seconds; and melt curve stage: 95ºC for 15 seconds, 55ºC for 60 seconds, temperature increment +0.3ºC, 95ºC for 15 seconds.

We quantified relative parasite intensity for two blood samples which were confirmed (using microsatellite loci sizing) to contain a minimum of one *P. falciparum* genotype (K10, IG14; single infections), two blood samples containing a minimum of two *P. falciparum* genotypes (SH79, SH25; double infections), and two blood samples containing a minimum of three *P. falciparum* genotypes (SH158, SH93; triple infections). Mean Cₜ values were calculated from 3 replicates per treatment. Delta Cₜ values were calculated (Delta Cₜ = Mean Cₜ,sample – Mean Cₜ,reference), and we plotted fold difference between target DNA and reference DNA (2⁻delta Cₜ) (Figure S3).
Alternative splicing of the *Anopheles gambiae* *Dscam* gene in diverse *Plasmodium falciparum* infections

**Results**

**Figure S3:** Fold difference in relative DNA quantity between human reference (GAPDH) and target (*Plasmodium falciparum*).

![Graph showing fold difference](image)

**Key:** +++; triple infection, ++; double infection, +; single infection.

**Acknowledgements**

We thank Lydia Chambers for carrying out the *Plasmodium falciparum* PCR detection assay.

**References**