Additional file 1: Tests on molecular markers associated with anti-malarial drug resistance

Targets

- The chloroquine transporter gene (Pfcr) codons 72-76;
- The multi-drug resistance gene 1 (Pfmdr1) codon 86;
- The dihydrofolate reductase gene (Pfdhfr) codons 51, 59, 108, 164;
- The dihydropteroate synthase gene (Pfdhps) codons 436, 437, 540, 581;
- The cytochrome b gene (Pfcytb) codon 268;
- The Kelch 13 (PfK13) gene: sequencing to screen for the presence of mutation

Methods

Tests were performed according the protocols proposed by Juliano et al. [a] for Pfcr; Crameri et al. [b] for Pfmdr1, Pfdhfr and Pfdhps; and Schwöbel et al. [c] for Pfcytb respectively. The sequencing of PfK13 was performed according to the protocol of Ariey et al. [d].

All the PCRs were run on Veriti® Thermal Cycler (Applied Biosystems®) and PCR products were visualized after electrophoresis using the QIAxcel® Advanced System (QIagen®) equipped with DNA Fast Analysis Kit (QIagen®). PCR products to be sequenced were purified with the QIAquick® PCR Purification Kit (Qiagen®), eluted in 30µL of elution buffer and frozen at -30°C prior sequencing in both directions. Sequencing preparation was done with the BigDye® Terminator v3.1 cycle sequencing Kit (Applied Biosystems®) and the respective oligonucleotide primers, purified with the BigDye® XTerminator™ Purification Kit (Applied Biosystems®) before being sequenced on a 3500xl Genetic Analyzer (Applied Biosystems®). For each step, kits and equipment were used according to the manufacturer’s recommendations.

Results

<table>
<thead>
<tr>
<th>Gene</th>
<th>Codon(s) tested</th>
<th>Results (mutant loci bolded)</th>
<th>Potential related drug resistance (R) / sensitivity (S)</th>
<th>GenBank Acc. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pfcr</td>
<td>72 to 76</td>
<td>CVIET</td>
<td>Chloroquine R</td>
<td>MF155939</td>
</tr>
<tr>
<td>Pfmdr1a</td>
<td>86</td>
<td>N</td>
<td>Amodiaquine S</td>
<td>MF155940</td>
</tr>
<tr>
<td>Pfdhfr</td>
<td>51, 59, 108, 164</td>
<td>IRNI</td>
<td>Sulfadoxine-pyrimethamine R</td>
<td>MF155941</td>
</tr>
<tr>
<td>Pfdhps</td>
<td>436, 437, 540, 581</td>
<td>SGKA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sulfadoxine-pyrimethamine R</td>
<td>MF155942</td>
</tr>
<tr>
<td>Pfcytb</td>
<td>268</td>
<td>Y</td>
<td>Atovaquone S</td>
<td>MF155943</td>
</tr>
<tr>
<td>PfK13</td>
<td>Sequence screening</td>
<td>No mutation</td>
<td>Artemisinin S</td>
<td>MF155944</td>
</tr>
</tbody>
</table>

<sup>a</sup> Due to limited sample amount, Pfmdr1 additional SNPs and variation in copy number were not tested.

<sup>b</sup> An additional non-synonymous mutation was detected: codon 484T
References


