

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

Targets

- The chloroquine transporter gene (*Pfcr1*) 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 (*Pfctb*) codon 268;
- The Kelch 13 (*PfK13*) gene: sequencing to screen for the presence of mutation

Methods

Tests were performed according to the protocols proposed by Juliano *et al.* [a]: for *Pfcr1*; Crameri *et al.* [b] for *Pfmdr1*, *Pfdhfr* and *Pfdhps*; and Schwöbel *et al.* [c] for *Pfctb* 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

Gene	Codon(s) tested	Results (mutant loci bolded)	Potential related drug resistance (R) / sensitivity (S)	GenBank Acc. No.
<i>Pfcr1</i>	72 to 76	CVIET	Chloroquine R	MF155939
<i>Pfmdr1</i> ^a	86	N	Amodiaquine S	MF155940
<i>Pfdhfr</i>	51, 59, 108, 164	IRNI	Sulfadoxine-pyrimethamine R Proguanil R	MF155941
<i>Pfdhps</i>	436, 437, 540, 581	SGKA ^b	Sulfadoxine-pyrimethamine R	MF155942
<i>Pfctb</i>	268	Y	Atovaquone S	MF155943
<i>PfK13</i>	Sequence screening	No mutation	Artemisinin S	MF155944

^a Due to limited sample amount, *Pfmdr1* additional SNPs and variation in copy number were not tested.

^b An additional non-synonymous mutation was detected: codon 484T

References

- a) Juliano JJ, Kwiek JJ, Cappell K, Mwapasa V, Meshnick SR. Minority-variant pfcr K76T mutations and chloroquine resistance, Malawi. *Emerg Infect Dis.* 2007;13:872-7.
- b) Crameri A, Marfurt J, Mugittu K, Maire N, Regös A, Coppee JY, et al. Rapid microarray-based method for monitoring of all currently known Single-Nucleotide Polymorphisms associated with parasite resistance to antimalaria drugs. *J Clin Microbiol.* 2007;45:3685-91.
- c) Schwöbel B, Alifrangis M, Salanti A, Jelinek T. Different mutation patterns of atovaquone resistance to *Plasmodium falciparum* *in vitro* and *in vivo*: rapid detection of codon 268 polymorphisms in the cytochrome b as potential *in vivo* resistance marker. *Malar J.* 2003;2:5.
- d) Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature.* 2014;505:50-5.