Efficacy models for antimalarial molecules

Background

Quantitative real-time PCR (qPCR) is now commonly used as a method to confirm diagnosis of malaria and primarily to differentiate recrudescence from re-infection, especially in clinical trials and in reference laboratories where precise quantification is critical. Although antimalarial drug discovery makes use of *in vivo* murine efficacy models, the use of molecular analysis from the models has been quite limited. The aim of our study was to develop qPCR as a methodology to support pre-clinical antimalarial models making use of material maintained in filter papers for qPCR analysis. Results were compared with traditional methods.

Methods

FTA technology (Whatman) is a rapid and safe method for extracting nucleic acids from infected blood. Peripheral blood samples from mice infected with *Plasmodium berghei*, *P. yoelii*, or *P. falciparum* were kept as frozen samples or as spots on FTA cards. The extracted genetic material from both types of samples was assessed for quantification by qPCR using sets of specific primers specifically designed for *Plasmodium* 18S rRNA, LDH, and CytB genes.

Results

The optimal conditions for nucleic acid extraction from FTA cards and qPCR amplification were set up, and were confirmed to be suitable for parasite quantification using DNA as template even after storage at room temperature

for as long as 26 months in the case of *P. berghei* samples and 52 months for *P. falciparum* and *P. yoelii*. The quality of DNA extracted from the FTA cards for gene sequencing and microsatellite amplification was also assessed.

Conclusions

This is the first study to report the suitability of FTA cards and further qPCR analysis to quantify parasite load in samples from *in vivo* efficacy models to support drug discovery processes.