

Development of insulated isothermal PCR for rapid on-site malaria detection

Abstract

Background

Detection of *Plasmodium* spp. is sometimes inconvenient especially in rural areas that are distant from a laboratory. In this study a portable diagnostic test of *Plasmodium* spp. was developed using insulated isothermal polymerase chain reaction (iiPCR) as an alternative approach to improve this situation.

Methods

A pair of universal primers and probe were designed to amplify and detect gene encoding 18S small sub-unit rRNA of *Plasmodium* spp using iiPCR method in a portable device, POCKIT™. The efficiency and detection limit of the assay were evaluated using quantitative real-time polymerase chain reaction (qPCR) approach before being subjected to testing in POCKIT™. Detection results of POCKIT™ were displayed as '+', '-' or '?' based on the fluorescence ratio after/before reaction. A total of 55 and 35 samples from malaria patients and healthy subjects, respectively, were screened to evaluate the feasibility of this newly designed iiPCR assay.

Results

The iiPCR assay allowed the detection of various species of *Plasmodium*, including those infecting humans (*Plasmodium falciparum*, *P. vivax*, *P. knowlesi*, *P. malariae*, *P. ovale*), monkeys, birds, and rodents. Efficiency of the assay achieved 96.9 % while the lower detection limit was ≥ 100 copies of plasmodial DNA. Specificity of the assay was assured as it could not detect human, bacterial and other parasitic DNA. Among the 55 clinical samples tested, 47 (85.4 %) of them were detected as positive by POCKIT™. Four (7.3 %) samples with fluorescence ratio after/before reaction of < 1.2 were reported as negative while another four (7.3 %) were ambiguously detected as they had fluorescence ratios between 1.2 and 1.3. The fluorescence ratio was not found to be

associated with the copy number of plasmodial DNA. This approach can only be considered as a qualitative method.

Conclusions

The portable iiPCR system may serve as an alternative approach for preliminary screening of malaria in endemic rural areas. The system may also be useful for detecting animal malaria in the field. Although it is not as quantitative as qPCR method, it is comparatively fast and easy to handle. It is believed that the POCKIT-iiPCR assay is able to achieve 100 % sensitivity if increased amount of DNA from each sample is used. The iiPCR assay can also be upgraded in future to detect multiple *Plasmodium* spp. at the same time by designing the specific primers and probes.