Development of high resolution melting analysis for the diagnosis of human malaria

Abstract

Molecular detection has overcome limitations of microscopic examination by providing greater sensitivity and specificity in *Plasmodium* species detection. The objective of the present study was to develop a quantitative real-time polymerase chain reaction coupled with high-resolution melting (qRT-PCR-HRM) assay for rapid, accurate and simultaneous detection of all five human *Plasmodium* spp. A pair of primers targeted the 18S SSU rRNA gene of the *Plasmodium* spp. was designed for qRT-PCR-HRM assay development. Analytical sensitivity and specificity of the assay were evaluated. Samples collected from 229 malaria suspected patients recruited from Sabah, Malaysia were screened using the assay and results were compared with data obtained using PlasmoNex^{TM}, a hexaplex PCR system. The qRT-PCR-HRM assay was able to detect and discriminate the five *Plasmodium* spp. with lowest detection limits of 1–100 copy numbers without nonspecific amplifications. The detection of *Plasmodium* spp. in clinical samples using this assay also achieved 100% concordance with that obtained using PlasmoNex^{TM}. This indicated that the diagnostic sensitivity and specificity of this assay in *Plasmodium* spp. detection is comparable with those of PlasmoNex^{TM}. The qRT-PCR-HRM assay is simple, produces results in two hours and enables high-throughput screening. Thus, it is an alternative method for rapid and accurate malaria diagnosis.