Molecular detection of human Plasmodium species in Sabah using PlasmoNextm multiplex PCR and hydrolysis probes real-time PCR

Abstract

BACKGROUND:

Malaria is a vector borne-parasitic disease transmitted through the bite of the infective female Anopheles mosquitoes. Five Plasmodium species have been recognized by World Health Organization (WHO) as the causative agents of human malaria. Generally, microscopic examination is the gold standard for routine malaria diagnosis. However, molecular PCR assays in many cases have shown improvement on the sensitivity and specificity over microscopic or other immunochromatographic assays.

METHODS:

The present study attempts to screen 207 suspected malaria samples from patients seeking treatment in clinics around Sabah state, Malaysia, using two panels of multiplex PCRs, conventional PCR system (PlasmoNex™) and real-time PCR based on hydrolysis probe technology. Discordance results between two PCR assays were further confirmed by sequencing using 18S ssu rRNA species-specific primers.

RESULTS:

Of the 207 malaria samples, Plasmodium knowlesi (73.4% vs 72.0%) was the most prevalent species based on two PCR assays, followed by Plasmodium falciparum (15.9% vs 17.9%), and Plasmodium vivax (9.7% vs 7.7%), respectively. Neither Plasmodium malariae nor Plasmodium ovale was detected in this study. Nine discrepant species identification based on both the PCR assays were further confirmed through DNA sequencing. Species-specific real-time PCR only accurately diagnosed 198 of 207 (95.7%) malaria samples up to species level in contrast to PlasmoNex™ assay which had 100% sensitivity and specificity based on sequencing results.

CONCLUSIONS:

Multiplex PCR accelerate the speed in the diagnosis of malaria. The PlasmoNex™ PCR assay seems to be more accurate than real-time PCR in the speciation of all five human malaria parasites. The present study also showed a significant increase of the potential fatal P. knowlesi infection in Sabah state as revealed by molecular PCR assays.