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Figure 1 Alignment of the small subunit rRNA gene derived from *P. vivax* (GenBank accession no.U83877) and *P. falciparum* (GenBank accession no.AF145334). Plas_F and Plas_R show position of forward and reverse PCR primers, respectively, as well as Plas_S shows position of sequencing primer. Target region and four asterisks in target region demonstrate the position that was used to identify and differentiate *P. vivax* and *P. falciparum* by pyrosequencing technique.

Figure 2 Pyrograms showing sequence analysis (SQA) of 24- and 21-base fragments of the small subunit rRNA gene of *P. vivax* (a-c) and *P. falciparum* (d-f) using pyrosequencing, respectively. Pyrosequencing was performed by addition of enzyme (E) and substrate (S), and four different nucleotides, enclosed in two squares in the pyrograms were analysed to discriminate *P. vivax* (a-c) and *P. falciparum* (d-f). The pyrograms represent the two different sequences of *P. vivax* (a-c): ACTAG-GCTTTGGATGAAAGATTTT and *P. falciparum* (d-f): ACTAGGTGTTGGATG-AAAGTG derived from positive control plasmids (a, d), patients at Mae-Sod (b, e) and Phang-Nga (c, f). Theoretical pyrogram patterns (top of each panel showing in histograms) and examples of raw data from pyrosequencing (bottom of each panel showing in peaks) were shown. Y-axis represents level of fluorescence emitted by the incorporation of a nucleotide base and x-axis represents total number of bases added at that point in time; A, T, C, G, nucleotide bases.



