menzies school of health research

STANDARD OPERATING PROCEDURE	PAGES 3
Title: **Detection of a common *pvmdr1* amplification using a breakpoint-specific PCR assay**	SOP Number: 1 Revision Number: 0 Effective Date: 8 September, 2016
Reason for Review:	Supersedes: Nil
Section:	Date for Review:

PURPOSE & PRINCIPLE

This SOP describes a standard PCR technique to detect the presence of *P. vivax* parasites with a 37.6 Kb amplification of *pvmdr1* (multidrug resistance 1 gene) seen commonly in western Thailand (Auburn *et al*. 2016). The SOP comprises three separate assays as illustrated in **Figure1** including two positive control assays (LF+LR and RF+RR), which should produce an amplicon in all *P. vivax* isolates with sufficient DNA, and a test assay (RF+LR), which should only produce an amplicon in *P. vivax* isolates with the common 37.6 Kb tandem *pvmdr1* amplification.

One copy:

Multiple copies:

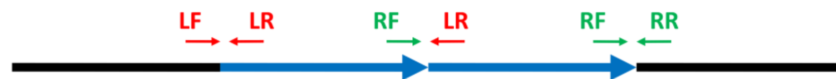

Figure 1. Schematic showing the orientation of the PCR primers to amplify control upstream (LF+LR; F=forward, R=reverse), control downstream (RF+RR) and amplification-specific (RF+LR) fragments.

REFERENCES

Auburn S, Serre D, Pearson RD, Amato R, Sriprawat K, To S, Handayuni I, Suwanarusk R, Russell, Drury E, Stalker J, Miotto O, Kwiatkowski DP, Nosten F, Price RN. Genomic Analysis Reveals a Common Breakpoint in Amplifications of the Plasmodium vivax Multidrug Resistance 1 Locus in Thailand. *J Infect Dis*. 2016 Jul 24.

- http://www.ncbi.nlm.nih.gov/pubmed/27456706

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PRIMER SEQUENCES

MDR1LF 5'-ACTGCGAAAGTCGCCTATTT-3'
MDR1LR 5'-TCATCGTGTGGCACATTTTT-3'
MDR1RF 5'-GGTGAAAAGGTCGAAGCAAA-3'
MDR1RR 5'-GGGACACGTTCCTCAGAAGT-3'

P. vivax reference strain: Salvador-1 (http://plasmodb.org/common/downloads/release-10.0/PvivaxSal1/fasta/data/PlasmoDB-10.0_PvivaxSal1_Genome.fasta)

REACTION MASTER MIX

- The same master mix and thermocycling conditions are used for all three assays.
- It is advisable to run the assays with positive and negative control samples.
- It is advisable to run samples with low parasite density in duplicate.

Reagent	Volume per reaction (µL)
DNA Template	1
10x buffer [Qiagen:15 mM MgCl2]	2
dNTPs (2 mM)	2
F primer (10 µM)	0.5
R primer (10 µM)	0.5
MgCl2 (25 mM)	0.4
5U Taq	0.3
PCR-grade water	13.3
Total	20

THERMOCYCLING

95°C, 3 min
35 cycles:
  95°C, 30 sec
  58°C, 40 sec
  72°C, 30 sec
72°C, 5 min

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GEL ELECTROPHORESIS

Run a minimum of 5 µl PCR product from each assay on a 1-2% agarose gel.

- The LF+LR assay produces an amplicon of ~408 bp.
- The RF+RR assay produces an amplicon of ~505 bp.
- The RF+LR assay produces an amplicon of ~580-600 bp.
- If the RF+LR assay is negative and the LF+LR and/or RF+RR assay is negative, the results are indeterminate.