

SUPPLEMENTAL DATA

Paper title: Fast and Sensitive Real-Time PCR Detection of Major Antiviral-Drug Resistance Mutations in Chronic Hepatitis B Patients by Use of a Predesigned Panel of Locked-Nucleic-Acid TaqMan Probes

TABLE S1 Cost estimation of real time PCR LNA probes assay compared to COLD-PCR combined Sanger DNA sequencing for detection of major elven NA-drug resistant mutations of HBV

Reagents and consumables for real time PCR LNA probes	Cost (US dollar)	Reagents and consumables for COLD-PCR & DNA sequencing	Cost (US dollar)
One reaction for each mt in the panel	0.66	One reaction for each fragment containing mt (P1 or P2)	9.88
<i>12.5 µL Real time PCR master mix^a</i>	<i>0.44</i>	<i>30 µL PCR master mix^b</i>	<i>1.06</i>
<i>Primers^c</i>	<i>-</i>	<i>Primers^c</i>	<i>-</i>
<i>Well of PCR 96-well plate & film^d</i>	<i>0.06</i>	<i>PCR tube^e</i>	<i>0.10</i>
<i>FAM and HEX labelled LNA probes^f</i>	<i>0.16</i>	<i>Reagents for 1-well electrophoresis: DNA ladder, DNA loading dye, buffer, agarose gel, and ethidium bromide^g</i>	<i>0.12</i>
		<i>PCR product purification^h</i>	<i>2.6</i>
		<i>Sanger DNA Sequencingⁱ</i>	<i>6.0</i>
One assay consisting of 11 reactions for 11 mt (x 11)	7.26	One assay consisting of 2 reactions for P1 and P2 fragments (x 2)	19.76
One assay with controls (6 samples, 1 negative control, 1 positive control of 5% mt/wt): 8 x 11 = 88 real-time PCR master mixes are prepared in a PCR 96-well plate	9.68	One assay with controls (6 samples and 1 negative control, 1 positive control of 1 representative mt of each fragment at 5% mt/wt): 8 x 2 =16 PCR master mixes are prepared in two PCR 8-tube strips (PCR purification and DNA sequencing are not applied for negative controls)	23.48

^aTOPreal™ qPCR 2XPreMIX (Enzynomics, Incheon, Korea): \$176 for 5 mL.

^b2X TOPsimple™ PreMIX-HOT (Enzynomics, Incheon, Korea): \$88 for 2.5 mL.

^cThe cost of primers is almost negligible.

^dFAM labelled LNA probe (IDT, IA, USA): \$620 for 8000 reactions; HEX labelled LNA probe (IDT, IA, USA): \$640 for 8000 reactions.

^eMicroAmp™ Fast 96-well reaction plate (Applied Biosystems, MA, USA): \$58.3 for 10 plates; MicroAmp™ Optical adhesive film (Applied Biosystems, MA, USA): \$33.8 for 100 films;

^fPCR tube (Thermo Scientific, MA, USA): \$100 for 1000 tubes.

^g100 bp DNA ladder (Enzynomics, Incheon, Korea): \$44 for 100 wells (8-tube PCRs were run in parallel with one ladder); 6X DNA loading dye (Enzynomics, Incheon, Korea): \$13.2 for 500 wells; the buffer, agarose gel, and ethidium bromide for one well were estimated to be \$0.02/well.

^hQIAquick PCR Purification Kit (Qiagen, Hilden, Germany): \$130 for 50 reactions.

ⁱCost of Sanger DNA sequencing service in Vietnam (either conducted in Vietnam or sent abroad for 1st base DNA sequencing in Singapore).