EVALUATION OF PRIMERS FOR THE DETECTION OF HIV-1 VIRUSES FROM KELANTAN BY USING REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)

by

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CERTIFICATE

This is to certify that the dissertation entitle "EVALUATION OF THE PRIMERS FOR THE DETECTION OF HIV-1 VIRUSES FROM KELANTAN BY USING REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)" is the bonafide record of research work done by Ms. Noor Hamizah binti Minal during the period from September 2012 to June 2013 under my supervision.

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SYMBOLS AND ABBREVIATIONS

°C	Degree Celcius
µg	Microgram
µL	Microlitre
bp	Base pair
BLAST	Basic Local Alignment Search Tool
cDNA	Complementary deoxyribonucleic acid
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	deoxynucleoside Triphosphate
EDTA	Ethylene Diamine Tetra Acetic acid
EtBr	Ethidium bromide
g	Gravity
HIV	Human immunodeficiency virus
kDa	kiloDalton
LTR	Long terminal repeat
mg	Miligram
$MgCl_2$	Magnesium Chloride
mM	miliMolar
NFW	Nuclease Free Water
ng	Nanogram
PCR	Polymerase Chain Reaction
qPCR	Qualitative Polymerase chain reaction
RNA	Ribonucleic acid
RNase	Ribonuclease
Rpm	Revolution per minute
RT	Reverse Transcriptase
RT-PCR	Reverse Transcription-Polymerase chain reaction
TBE	Tris Borate EDTA
U	Unit
UV	Ultraviolet
ρM	Picomolar

ABSTRACT

Human immunodeficiency virus type 1 (HIV-1) has been recognized as the causative agent of acquired immunodeficiency syndrome (AIDS). Approximately 42 million people carrying the virus at present, but its case fatality rate is close to 100%, making it an infection of devastating ferocity. Since the evolutionary change of the virus is at rapid rate, it imposes a challenge on HIV diagnostic development, vaccine development, antiretroviral drug sensitivity and drug resistance. Rapid and sensitive methods for the detection of HIV-1 viruses would be valuable in controlling this disease. Currently, nucleic acid amplification tests such as reverse transcription polymerase chain reaction (RT-PCR) and real-time PCR (rtPCR) are widely used in detecting HIV-1 viruses. These tests are commonly applied in blood donation screening and early detection of HIV-1 in infants. High sequence variations of the HIV-1 viruses from Kelantan have led to the failure to obtain the amplification product using published primers. Therefore, in this study, a few set of primers were designed based on the sequence alignments of published HIV-1 viruses from Malaysia and Kelantan which were retrieved from NCBI genebank. The primers were evaluated on 30 confirmed HIV-1 positive samples from Kelantan, Malaysia. HIV LTRF2 and HIV LTR2 were chosen as the best primers to detect these viruses since they were successfully amplified all the 30 samples used in this study. The primers were further evaluated their sensitivity and specificity against 3 viruses which are Japanese encephalitis (JE), Chikungunya and Western encephalitis virus (WEE) where no expected band yielded. These primers were sensitive and specific enough in detecting HIV-1 viruses from Kelantan. They were further evaluated for use in the real-time PCR and can be used in this test. Thus, these primers could be potentially use for the future diagnosis of HIV-1 viruses from Kelantan either by conventional or real time PCR.

ABSTRAK

"Human Immunodeficiecy Virus" (HIV) jenis 1 telah dikenal pasti sebagai agen penyebab kepada Sindrom Kurang Daya Tahan Penyakit (AIDS). Sehingga kini dianggarkan hampir 42 juta orang pembawa virus ini, dengan kadar kematiannya hampir kepada 100%, menjadikannya sebagai satu jangkitan yang boleh membunuh. Memandangkan perubahan evolusi virus tersebut pada kadar yang sangat cepat, ini menimbulkan satu cabaran dalam pembangunan diagnostik dan vaksin, sensitiviti ubat antiretroviral dan ketahanan terhadap ubat. Ujian amplifikasi asid nukleik merupakan pendekatan yang digunakan secara meluas dalam mengesan virus HIV-1. Pendekatan ini juga banyak dilaksanakan untuk memeriksa darah yang didermakan dan pengesanan awal HIV-1 dalam bayi. Variasi yang tinggi dalam jujukan virus HIV-1 yang terdapat di Kelantan telah menyebabkan virus tersebut tidak dapat dikesan dalam proses PCR dengan menggunakan primer rujukan yang dihasilkan oleh kajian sebelum ini. Justeru, beberapa set primer telah direka dan dinilai keatas 30 sampel positif HIV-1 dari Kelantan. Dalam kajian ini, HIV LTRF2 dan HIV LTR2 telah dipilih sebagai primer yang terbaik untuk mengesan virus HIV-1 yang terdapat di Kelantan memandangkan primer-primer ini berjaya mengamplifikasikan kesemua 30 sampel yang digunakan dalam kajian ini. Sensitiviti dan spesifisiti primer ini kemudian dinilai keatas tiga jenis virus lain iaitu Japanese encephalitis (JE), Chikungunya dan 'Western equine encephalitis virus' (WEE) di mana tiada amplifikasi yang berlaku. Primer ini didapati sensitif dan spesifik dalam pengesanan virus HIV dari Kelantan. Primer ini seterusnya diuji sama ada ia boleh digunakan dalam 'real-time' PCR. Hasil yang diperolehi menunjukkan primer-primer ini boleh digunakan dalam 'real-time' PCR. Oleh itu, primer ini disahkan boleh untuk digunakan untuk diagnosa virus HIV dari Kelantan pada masa akan datang samada secara konvensional PCR atau 'real-time' PCR.