

## TABLE OF CONTENTS

	Page
<b>ACKNOWLEDGEMENTS</b>	iii
<b>ABSTRACT</b>	v
<b>LIST OF TABLES</b>	xii
<b>LIST OF FIGURES</b>	xiv
<b>ABBREVIATIONS</b>	xvi
<b>CHAPTER 1: INTRODUCTION</b>	1
Objectives	3
<b>CHAPTER 2: LITERATURE REVIEW</b>	4
1. The epidemic of HIV-1	4
2. Human immunodeficiency virus type 1	4
3. Laboratory diagnostic for HIV-1 infection	11
4. Laboratory monitoring for HIV-1 infection	14
5. Real-time polymerase chain reaction	16
6. Quantitative real-time PCR	20
7. PCR inhibitors	21

<b>CHAPTER 3: SYUDY DESING, METERIALS AND METHODS</b>	22
1 Study design	22
2 Materials	23
2.1 Plasma samples	23
2.2 Primers and probes	23
2.3 Plasmid vectors	24
2.4 Commercial kit	28
3 Methods	28
3.1 Viral RNA extraction	28
3.2 <i>E. coli</i> competent cell preparation	29
3.3 The HIV-1 <i>gag</i> gene amplification from pro-viral DNA	29
3.4 DNA purification from agarose gel	30
3.5 Internal system construction using splicing-overlapping extension PCR (SOE-PCR)	30
3.6 The PCR cloning of standard HIV-1 <i>gag</i> gene and HIV-1 internal system control	32
3.7 Colony PCR for selection of transformant	33
3.8 Plasmid purification and confirmation	33
3.9 <i>In vitro</i> transcription for HIV-1 <i>gag</i> RNA and internal system control RNA production	34
3.10 Determination of HIV-1 RNA transcript	35
3.11 Real-time PCR for quantitation of HIV-1 RNA	36
3.12 Optimization of HIV-1 RNA internal system control	37

3.13 Standard curve construction	37
3.14 The reproducibility of the assay	38
3.15 Viral RNA quantitation kit used in this study	38
3.16 Statistical analysis	38
<b>CHAPTER 4: RESULTS</b>	39
1. HIV-1 <i>gag</i> gene and internal system control construction	39
2. <i>In vitro</i> transcription for HIV-1 <i>gag</i> RNA and internal system control synthesis	44
3. Standard curve construction and test limitation	45
4. Optimization of HIV-1 RNA internal system control	45
5. Assay reproducibility	49
6. Determination of HIV-1 positive plasma samples using validated real-time PCR	51
7. The correlation and agreement between the validated assay and standard reference kit determination	67
<b>CHAPTER 5: DISCUSSION</b>	71
<b>CHAPTER 6: CONCLUSION</b>	76
<b>REFERENCES</b>	77
<b>APPENDICES</b>	85
<b>CURRICULUM VITAE</b>	90

## LIST OF TABLES

Table		Page
1	The nucleotide sequence of primers and probes used in this study	25
2	List of commercial kits used in this study	28
3	Reproducibility determination of intra-run assay (n=6) using the validated real-time PCR	50
4	Reproducibility determination of inter-run assay (n=8) using the validated real-time PCR	50
5	Determination of HIV-1 positive plasma samples using validated real-time PCR and comparison with reference method	53
6	The log <sub>10</sub> values of HIV-1 positive plasma samples determined by the validated and reference real-time PCR assay and the log <sub>10</sub> difference between both methods (40-100 copies/ml)	54
7	The log <sub>10</sub> values of HIV-1 positive plasma samples determined by the validated and reference real-time PCR assay and the log <sub>10</sub> difference between both methods (100-1,000 copies/ml)	56

- |    |   |    |
|----|---|----|
| 8  | The log <sub>10</sub> values of HIV-1 positive plasma samples determined by the validated and reference real-time PCR assay and the log <sub>10</sub> difference between both methods (1,000-10,000 copies/ml)      | 59 |
| 9  | The log <sub>10</sub> values of HIV-1 positive plasma samples determined by the validated and reference real-time PCR assay and the log <sub>10</sub> difference between both methods (10,000-100,000 copies/ml)    | 61 |
| 10 | The log <sub>10</sub> values of HIV-1 positive plasma samples determined by the validated and reference real-time PCR assay and the log <sub>10</sub> difference between both methods (100,000-1,700,000 copies/ml) | 63 |

## LIST OF FIGURES

Figure		Page
1	The schematic of Human immunodeficiency virus type 1 (HIV-1) structure	6
2	The life cycle of HIV-1 in host cell	8
3	Phases of PCR amplification curve	17
4	The study design used in this study	22
5	The primers and probes binding site on HIV-1 genome used in this study	24
6	Map and multiple cloning site of pGEM <sup>®</sup> -TVector Systems	26
7	Map and multiple cloning site of pDrive cloning vector	27
8	Illustration of internal system control construction by Splice overlapped extension PCR (SOE-PCR)	31
9	1.5% agarose gel electrophoresis of HIV-1 <i>gag</i> gene amplification by PCR	40
10	The colony-PCR amplification for detection of a positive clone	41
11	Internal system control construction by Splice overlapped extension-PCR (SOE-PCR)	42

12	Chromatogram of direct sequencing for integrity determination of HIV-1 <i>gag</i> and internal system control sequence	43
13	<i>In vitro</i> transcription for HIV-1 <i>gag</i> RNA and HIV-1 internal system control synthesis	44
14	External standard curve for HIV-1 virus quantitation using validated assay	46
15	The cut-off determination of internal system control RNA (IC-RNA)	47
16	Amplification curve obtained from internal system control RNA (IC-RNA) optimization.	48
17	Illustration of the fluorescent signal curve obtained from sero-negative plasma samples using validated real-time PCR assay	52
18	The demonstration of fluorescent signal obtained from 15 positive plasma samples after examined with validated real-time PCR assay.	66
19	The correlation analysis of validated and reference methods	68
20	The correlation analysis of validated and reference methods	69
21	An agreement analysis of reference and validated assay	70
22	The fluorescent signaling curve of 5 positive plasma samples that was pre-concentrated by high speed centrifugation and re-analyzed by real-time PCR.	75

## ABBREVIATIONS

$\mu$ l	microliter
AIDS	Acquired Immune Deficiency Syndrome
bp	Base pair
CD	Cluster of differentiation
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphates
<i>E.coli</i>	<i>Escherichia coli</i>
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus type-1
kDa	Kilodalton
LB	Luria-Bertani
Ig	Immunoglobulin
M	Molar
min	minute
OD	Optical density
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
rpm	round per minute



RT-PCR	Reverse transcription-polymerase chain reaction
sec	second
SOE-PCR	Splicing by overlapping extension PCR
UNAIDS	The United Nations Programme on AIDs
WHO	World Health Organization

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright © by Chiang Mai University  
All rights reserved