TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENT	iii
ABSTRAC IN ENGLISH	iv
ABSTRAC IN THAI	vi
LIST OF TABLES	xii
LIST OF ILLUSTRATIONS	xiv
ABBREVATIONS	XV
CHAPTER I: INTRODUCTION	1
OBJECTIVE	8
CHAPTER II: LITERATURE REVIEWS	9
Neisseria gonorrhoeae	9
Biology	9
Life cycle and Pathogenesis	11
Epidemiology	17
Clinical manifestations	20
Laboratory diagnosis	22
Treatment	27
Chlamydia trachomatis	28
Biology	28
Chlamydial development cycle	31
Epidemiology	32
Clinical manifestations and Matter University	34
Laboratory diagnosis	42
Treatment	49
Polymerase Chain Reaction (PCR) Amplification	49

Multiplex PCR	52
Real Time PCR	53
Cycle threshold	56
CHAPTER III: RESEARCH DESIGN, MATERAILS AND METHODS	59
1. Research design	59
2. Materials and methods	
2.1 Extraction of recombinant plasmids containing N. gonorrhoeae (pJD1)	60
and C. trachomatis (pCHL1) cryptic plasmid DNA from transformed	
E. coli	
2.2. In-house Taqman-based Real Time PCR	
2.2.1 In-house Taqman-based multiplex Real Time PCR for	62
detection of <i>N. gonorrhoeae</i> and <i>C. trachomatis</i> plasmid	
DNA CONSTRUCTION	
2.2.2 In-house Taqman-based Real Time PCR for detection of	63
N. gonorrhoeae porin A pseudogene DNA	
2.3. Determination of the optimal concentration of primers and probes.	
2.3.1 In-house Taqman-based multiplex Real Time PCR for	65
detection of N. gonorrhoeae and C. trachomatis plasmid	
DNA	
2.3.2 In-house Taqman-based Real Time PCR for detection of	65
N. gonorrhoeae porin A pseudogene DNA	
2.4. Determination sensitivity of In-house Taqman-based Real Time PCR.	
2.4.1 In-house Taqman-based multiplex Real Time PCR for	66
detection of <i>N. gonorrhoeae</i> and <i>C. trachomatis</i> plasmid	
DNA	
2.4.2 In-house Taqman-based Real Time PCR for detection of	66
N. gonorrhoeae porin A pseudogene DNA	
2.5. Determination of the specificity of In-house Taqman-based	66
multiplex Real Time PCR for detection of N. gonorrhoeae and	
C. trachomatis and In-house Taqman-based Real Time PCR for	
detection of N.gonorrhoeae porin A pseudogene.	

ix

2.6. Comparison between In-house Taqman-based multiplex Real Time	68
PCR and Roche Multiplex AMPLICOR CT/NG PCR method in	
detection of N. gonorrhoeae and C. trachomatis methods urine	
samples .	
2.7. Confirmation assay for N. gonorrhoeae by using In-house	70
Taqman-based Real Time PCR detecting Porin A pseudogene	
2.8. Confirmation assay of C. trachomatis cryptic plasmid positive results	70
by detecting Major Outer Membrane Protein gene using conventional	
PCR method.	
2.9. Beta-globuin gene testing using conventional PCR method.	70
CHAPTER IV: RESULTS	72
1. Extraction of recombinant plasmid containing C. trachomatis (pCHL1)	72
and N. gonorrhoeae (pJD1) DNA from the transformed E. coli	
2. Determination optimal concentration of probe and primer used in the assay.	
2.1. In-house Taqman-based multiplex Real Time PCR for detection of	73
N. gonorrhoeae and C. trachomatis plasmid DNA	
2.2. In-house Taqman-based Real Time PCR for detection of N. gonorrhoeae	75
Porin A pseudogene DNA	
3. Determination the sensitivity of In-house Taqman-based Real Time PCR	
3.1. In-house Taqman-based multiplex Real Time PCR for detection of	76
N. gonorrhoeae and C. trachomatis plasmid DNA	_
3.2. In-house Taqman-based Real Time PCR for detection of Porin	78
A pseudogene DNA of <i>N. gonorrhoeae</i>	
4. Determination specificity of In-house Taqman-based multiplex Real Time	79
PCR for detection of N. gonorrhoeae and C. trachomatis and In-house	
Taqman-based Real Time PCR for detection of <i>N. gonorrhoeae</i> porin A	
pseudogene DNA	

X

4.1. Specificity testing by using computer software.	79	
4.2. Specificity testing by performing the real time PCR amplification		
with related micro organisms.		
5. Comparison of In-house Taqman-based multiplex Real Time PCR with	83	
Roche Multiplex AMPLICOR CT/NG PCR assay in detecting		
N. gonorrhoeae and C. trachomatis from urine samples.		
CHAPTER V: DISCUSSION AND CONCLUSION	89	
REFERENCE	94	
APPENDICES		
APPENDIX A: Equipments	112	
APPENDIX B: Chemicals and reagents	113	
APPENDIX C: Reagents and buffer preparation	114	
CIRRICULUM VITAE		
The second second		
MIL TERS		
UNIVE.		

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

LIST OF TABLES

Table	Page
1. Sequence of oligonucleotide primers and probe used in In-house	64
Taqman-based Real Time PCR for detection of N. gonorrhoeae	
Porin A pseudogene	
2. List of non-pathogenic and pathogenic organisms from clinical	68
isolates and standard strains used in specificity testing by	
Real Time PCR	
3. The results of recombinant plasmid DNA preparation	73
4. Cycle threshold (Ct) of Real Time PCR reaction for detecting	74
N. gonorrhoeae plasmid DNA under varying primer and probe	
concentrations.	
5. Results of Real Time PCR in detecting C. trachomatis plasmid	75
DNA by varying primer and probe concentrations.	
6. Demonstration the cycle threshold (Ct) results of Real Time	76
PCR reaction in detecting N. gonorrhoeae Porin A pseudogene	
DNA by varying primer and probe concentrations.	
7. Results of In-house Taqman-based multiplex Real Time in	77
detection of N. gonorrhoeae recombinant plasmid DNA	
8. Results of In-house Taqman-based multiplex Real Time in	78
detection of <i>N. gonorrhoeae</i> recombinant plasmid DNA	ΚIJ
Copyright [©] by Chiang Mai Univer	sity
All rights reserv	e d

xii

- Results of In-house Taqman-based Real Time PCR for Porin A
 pseudogene DNA of *N. gonorrhoeae*.
- 10. List of DNA sequences from microorganisms used as target DNA
 81 for specificity testing of primers and probes by using computer software.
- 11. Demonstration list of non-pathogenic and pathogenic micro organisms 83 used for specificity testing by real time PCR assay.
- 12. Comparison between In-house Taqman-based multiplex Real Time
 PCR results and Roche Multiplex AMPLICOR CT/NG PCR results
 detection *N. gonorrhoeae* in urine samples.
- 13. Comparison between In-house Taqman-based multiplex Real Time88PCR results and Roche Multiplex AMPLICOR CT/NG PCR resultsdetection C. trachomatis in urine samples.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

A MAI

LIST OF FIGURES

Figure 018191	Page
1. Illustration of N. gonorrhogge life cycle and pathogenesis	12
2. Illustration of the life evole nethogeneois of C tracker atia	22
2. Indistration of the file cycle pathogenesis of C. <i>trachomatis</i>	52
3. Illustration of the Polymerase Chain Reaction procedure	51
4. Diagram of multiplex PCR with probe detection systems	52
5. The principle of TaqMan probes in real-time PCR	55
6. Shows a representative amplification plot and defines the terms used	56
in the quantitation analysis	
7. Illustration of the location of primers and probes used in the	° 62
In-house Taqman-based multiplex Real Time PCR for detection of	
N. gonorrhoeae and C. trachomatis plasmid DNA assay	
8. Illustration the location of primers and probe specific to	64
N. gonorrhoeae porin A pseudogene used in In-house Taqman-based	
Real Time PCR for confirmation of the detection of N. gonorrhoeae	
9. Illustration the PCR amplified product of N. gonorrhoeae (pJD1) and	l 72
C. trachomatis (pCHL1) plasmid DNA	
10. Illustrated of the result from specificity testing by using real time	82
PCR assays.	
11. Illustration of conventional PCR detection of the MOMP gene	85
in the C. trachomatis.	K1
12. Detecting added beta-human globulin DNA by conventional PCR.	85
Copyright [©] by Chiang Mai Univer	rsity
All rights reserv	ec

	ABB	REVIATIONS
	an Sh	246
	β	beta
	°C	degree Celsius
9	μg	microgram
5	μΙ	microliter
6	Α	adenine
	ag	attogram
502	bp	base pair
500	C	cytosine
	CDC	Centers for Disease Control and Prevention
G	Ct	cycle threshold
	dATP	deoxyadinosine triphosphate
T,	dCTP	deoxycytosine triphosphate
Y.	DFA	direct immunofluorescence assays
	dGTP	deoxyguanine triphosphate
	DGI	disseminated gonococcal infection
	DNA	deoxyribonucleic acid
	dNTP	deoxynucleotide triphosphate
ດິມສິກຄິ	dTTP	deoxythymidine triphosphate
adalib	EB	elementary body
Copyright	FA by Ch	enzyme immunoassays fluorescent antibody
All r	_G ^{fg} g h t	guanine SETVE
	g	gram
	HIV	Human Immunodeficiency Virus
	IgA	immunoglobulin A

	IL	inter leukin
	Inc	inclusion membrane
	Lbp	Lactoferrin-binding protein
	LCR	ligase chain reaction
	LGV	lymphogranuloma venereum
	LOS	lipooligosaccharide
	LPS	lipopolysaccharide
	mAbs	monoclonal antibodies
	ml	milliter
	mM	millimolar
300	MOMP	major outer membrane protein
502	MSM	Men Sex with Men
208-	MTM	modified Thayer-Martin
	MW	molecular weight
	NAATs	Nucleic acid amplification tests
	Ng	nanogram
T I	NGU	nongonococcal urethritis
	NHS	nonimmune normal human serum
	O.D.	optical density
	Opa	opacity-associated
	PCR	polymerase chain reaction
	pg	picogram
ลิสสิทธิ	PID	pelvic inflammatory disease
ciocino	PMNs	polymorphonuclear leukocytes
Copyright	Por by C	poring Mai University
	RB	reticulate body
AII r	Rmp II	reduction-modifiable protein
	RNA	ribonucleic acid
	rRNA	ribosomal RNA
	SDA	displacement amplification



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

xvii