

**DETERMINATION OF LIPL32 EPITOPE OF  
LEPTOSPIROSIS VIA *in silico* METHOD AND  
ELISA**



**MOHD ISKANDAR BIN JUMAT**

**UMS**  
UNIVERSITI MALAYSIA SABAH

**BIOTECHNOLOGY RESEARCH INSTITUTE  
UNIVERSITI MALAYSIA SABAH  
2018**

**DETERMINATION OF LIPL32 EPITOPE OF  
LEPTOSPIROSIS VIA *in silico* METHOD AND  
ELISA**

**MOHD ISKANDAR BIN JUMAT**



**UMS**  
UNIVERSITI MALAYSIA SABAH

**THIS THESIS IS SUBMITTED IN  
FULLFILLMENT FOR THE DEGREE OF MASTER  
OF SCIENCE**

**BIOTECHNOLOGY RESEARCH INSTITUTE  
UNIVERSITI MALAYSIA SABAH  
2018**

**UNIVERSITI MALAYSIA SABAH**  
BORANG PENGESAHAN STATUS TESIS

JUDUL: DETERMINATION OF LIPL32 EPITOPE OF LEPTOSPIROSIS VIA *in silico* METHOD AND ELISA

IJAZAH: IJAZAH SARJANA (BIOTEKNOLOGI)

Saya Mohd Iskandar bin Jumat, Sesi 2015-2018, mengaku membenarkan tesis Sarjana ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis ini adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.

Sila tandakan (√):



SULIT

TERHAD

TIDAK TERHAD

(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA 1972)

(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

UNIVERSITI MALAYSIA SABAH

Disahkan Oleh,

\_\_\_\_\_  
MOHD ISKANDAR BIN JUMAT  
MZ1511006T

\_\_\_\_\_  
(Tandatangan Pustakawan)

TARIKH : 28 Februari 2018

\_\_\_\_\_  
(Dr Zarina Binti Amin)  
Penyelia

\_\_\_\_\_  
(Dr Rafidah Binti Othman)  
Penyelia Bersama

\_\_\_\_\_  
(Prof. Madya Dr Kenneth Francis Rodriguez)  
Penyelia Bersama

## DECLARATION

I hereby declare that the material in this thesis is my own except quotations, equations, summaries and references, which have been dully acknowledged.

5 Mac 2018

---

(MOHD ISKANDAR BIN JUMAT)



UMMS  
UNIVERSITI MALAYSIA SABAH

MZ1511006T

**CERTIFICATION**

**NAME : MOHD ISKANDAR BIN JUMAT**  
**MATRIC NO : MZ1511006T**  
**TITLE : DETERMINATION OF LIPL32 EPITOPE OF LEPTOSPIROSIS  
VIA *in silico* METHOD AND ELISA**  
**DEGREE : MASTER OF SCIENCE (BIOTECHNOLOGY)**  
**VIVA DATE : 23 JANUARY 2018**

**CERTIFIED BY :**

- |  |           |
|--|-----------|
| 1. SUPERVISOR (CHAIRPERSON)                      | Signature |
| Dr Zarina binti Amin                             | _____     |
| 2. COMMITTEE MEMBER 1                            |           |
| Associate Professor Dr Daisy Vanitha John        | _____     |
| 3. COMMITTEE MEMBER 2                            |           |
| Dr Rafidah binti Othman                          | _____     |
| 4. COMMITTEE MEMBER 3                            |           |
| Associate Professor Dr Kenneth Francis Rodrigues | _____     |

## **ACKNOWLEDGEMENT**

First of all, I would like to express my highest gratitude to Allah S.W.T for giving me strength and wisdom to complete my master study here in University Malaysia Sabah and also to both my parents and family for supporting me throughout my studies. I would also like to thank my supervisor Dr Zarina Amin and the supervisory committee Associate Professor Dr Daisy Vanitha John, Dr Rafidah Othman and Associate Professor Dr Kenneth Francis Rodriguez for guiding me and providing me with research grant for my research. Highest appreciation to Madam Rashidah from Public Health Laboratory and Dr Normah Yusop from Veterinary Department Kota Kinabalu for providing me with serum samples. Lastly, I would like to thank all the staff especially lab assistant Pn Azian, En Emran, En Mony, En Nordin and my fellow colleague Yuszniatyati Yaakob, Noraini Philip and Lia Natasha for helping me with my work.



NAME : MOHD ISKANDAR BIN JUMAT  
DATE : 5 FEBRUARY 2018

UMS  
UNIVERSITI MALAYSIA SABAH

## ABSTRACT

Leptospirosis is a predominant zoonotic disease caused by a pathogenic strain of *L. interrogans*. Diagnosis of leptospirosis includes microscopic agglutination test (MAT assay) and enzyme-linked immunosorbent (ELISA) which utilizes 6 designated outer membrane protein (OMP). Of these LipL32 is found to be most useful. However current prediction of the LipL32 epitope has been largely depend on empirical rather than *in silico* methods. In addition, currently no known reports of LipL32 epitope prediction using more than one bioinformatic algorithm. In this study, full length LipL32 protein was analyzed using three bioinformatic algorithms, which were the Kyte and Doolittle hydropathy scale, the recognition factor and the Kolaskar and Tangaokar propensity scale. The application of these three algorithm overlaps resulted in the recognition of three antigenic regions namely the NrLipL32 (amino acid sequence Ala<sub>21</sub>-Glu<sub>122</sub>), the intermediate LipL32 (amino acid sequence Pro<sub>144</sub>-Glu<sub>171</sub>) and the CrLipL32 (amino acid sequence Thr<sub>200</sub>-Ser<sub>261</sub>) fragments. Investigation for sensitivity and specificity among the fragments via ELISA indicated that CrLipL32 has the highest sensitivity (IgM 73.3% and IgG 60%) indicating to be the immunodominant epitope of LipL32. In conclusion, this study demonstrated that CrLipL32 is the immunodominant fragment of LipL32.

## **ABSTRAK**

*Leptospirosis ialah merupakan penyakit zoonotik utama yang disebabkan oleh Leptospira strain patogenik. Diagnosa leptospirosis termasuklah teknik MAT dan ELISA dimana ia menggunakan 6 protien membran luar (PML) yang terpilih. Diantara ini, LipL32 telah terbukti sebagai sangat berguna. Walaubagaimanapun, pada masa ini ramalan terhadap epitop LipL32 telah banyak menggunakan kaedah empirikal berbanding in siliko. Tambahan lagi, hingga masa kini tiada laporan menunjukkan meramal epitop LipL32 menggunakan lebih daripada satu algoritma bioinformatik. Dalam kajian ini, panjang penuh protein LipL32 telah dianalisa menggunakan tiga algoritma bioinformatik iaitu skala hydropati Kyte dan Doolittle, skala faktor rekognasi, dan skala faktor kecenderungan Kolaskar dan Tangaokar. Melalui hasil pertindihan aplikasi tiga algoritma ini, tiga bahagian antigen dapat dikenal pasti iaitu NrLipL32 (urutan amino asid Ala<sub>21</sub>-Glu<sub>122</sub>), pertengahan LipL32 (urutan amino asid Pro<sub>144</sub>-Glu<sub>171</sub>) dan CrLipL32 (urutan amino asid Thr<sub>200</sub>-Ser<sub>261</sub>). Kajian terhadap kesensitivitian dan kespesifisitian antara bahagian tadi melalui ELISA, menunjukkan CrLipL32 mempunyai sensitiviti tertinggi (IgM 73.3% dan IgG 60%) dan merupakan juga sebagai epitop immunodominan LipL32. Konklusinya, ini menunjukkan CrLipL32 adalah merupakan bahagian immunodominan epitop LipL32.*



## TABLE OF CONTENTS

	Pages
<b>TITLE</b>	i
<b>DECLARATION</b>	ii
<b>CERTIFICATION</b>	iii
<b>ACKNOWLEDGEMENT</b>	iv
<b>ABSTRACT</b>	v
<b>ABSTRAK</b>	vi
<b>TABLE OF CONTENTS</b>	vii-x
<b>LIST OF TABLES</b>	xi
<b>LIST OF FIGURES</b>	xii
<b>LIST OF ABBREVIATIONS</b>	xiii
<b>LIST OF SYMBOLS</b>	xiv
<b>LIST OF APPENDICES</b>	xv
<b>CHAPTER 1 : INTRODUCTION</b>	1-4
1.1 Background of study	1-3
1.2 Problem statement	3
1.3 Hypothesis	3
1.4 Objectives	4
<b>CHAPTER 2 LITERATURE REVIEW</b>	5-24
2.1 History of leptospirosis	5
2.2 <i>Leptospira interrogans</i> the etiological agent	6-8
2.3 Transmission of leptospirosis	8-10
2.4 Symptoms of leptospirosis	10-11
2.5 Treatment and Prevention	12
2.6 Diagnostic measures of leptospirosis	12-13
2.7 Recombinant outer membrane protein (rOMP) utilize in ELISA to diagnose Leptospirosis	13-16
a. Leptospiral immunoglobulin-like protein (LigA and LigB)	15
b. Transmembrane protein (OMPL1)	15
c. Lipoprotein (LipL41, LipL21, LipL32)	15-16

	Pages
2.8 Epitope	17-23
2.8.1 Experimental approaches in epitopes identification	18
2.8.2 Epitope prediction using <i>in silico</i> methods	18-21
2.8.3 LipL32 epitope	21
2.8.4 Some chosen bioinformatic tools for prediction of LipL32 linear epitopes	22-23
a. Kyte and Doolittle hydrophathy plot	22
b. Kolaskar and Tangaokar propensity scale of amino acid	22
c. Recognition factor scale of amino acid	23
2.9 Detection of Leptospira-specific IgG and IgM antibodies in ELISA	23-24
2.10 Treatment and prevention of leptospirosis	24
<b>CHAPTER 3 : MATERIALS AND METHODOLOGY</b>	25-45
3.1 List of serum samples, growth media, bacterial culture and oligonucleotide primers	25-30
3.1.1 List of serum samples	25-29
3.1.2 Growth media and bacterial culture	30
3.1.3 Oligonucleotide primers	30
3.2 Determination of LipL32 antigenic regions via <i>in silico</i> prediction	31
3.3 Development of full length rLipL32 and its antigenic fragments	31-38
3.3.1 Cloning, expression and purification of full length rLipL32 protein	31-35
3.3.1.1 Generation of synthetic <i>lip32</i> gene and reconstitution of codon optimized synthetic <i>lip32</i> gene	31
3.3.1.2 Preparation of competent <i>E.coli</i> cells and transformation of <i>lip32</i> gene in <i>E.coli</i> BL21 (DE3)	32

	Pages
3.3.1.3 Screening of positive transformants for <i>lip/32</i> inserts using colony PCR	33-34
3.3.1.4 Expression and purification of rLipL32 protein	34-35
3.3.2 Development of LipL32 antigenic fragments	35
3.3.3 Analyzing full length rLipL32 and its antigenic fragments	35-38
3.3.3.1 Protein estimation (Bradford assay)	35-36
3.3.3.2 SDS PAGE of full length rLipL32, CrLipL32 and NrLipL32 fragments	36-38
A) Preparation of SDS PAGE resolving gel	36
B) Preparation of SDS PAGE stacking gel	37
C) Sample preparation and running SDS PAGE	37-38
3.3.3.3 LC-MS/MS	38
3.4 Comparison of LipL32 antigenic determinant in ELISA using MAT positive and negative serum samples	38-45
3.4.1. ELISA of MAT positive and negative human serum samples using full length rLipL32	38-40
3.4.2. Comparison of LipL32 fragments in ELISA	41-42
3.4.3. ELISA of RCPAQAP human serum samples using full length rLipL32	43-44
3.4.4. Calculation of sensitivity and specificity for ELISA	45
<b>CHAPTER 4 : RESULT</b>	<b>46-53</b>
4.1 Sample collection	46
4.2 Determination of LipL32 antigenic regions	46-51
4.2.1 Determination of LipL32 antigenic regions using Kyte and Doolittle hydrophathy scale	46-47
4.2.2 Prediction of LipL32 antigenic regions using recognition factor scale	48
4.2.3 Prediction of LipL32 antigenic regions using Kolaskar and Tangaokar propensity scale	49-50

	Pages
4.2.4 LipL32 antigenic regions based on combination of the predicted algorithms	50-51
4.3 Production of full length rLipL32, CrLipL32 and NrLipL32	52-57
4.3.1 Production of full length recombinant LipL32 (rLipL32)	52-54
4.3.1.1 Colony PCR	52
4.3.1.2 Protein expression and purification	53
4.3.2 Analysis of full length rLipL32 and its antigenic determinant (NrLipL32, CrLipL32 and intermediate LipL32)	54-57
4.3.2.1 Protein estimation using Bradford assay	54-55
4.3.2.2 Yield of full length rLipL32, NrLipL32, and CrLipL32 from 1L of expressed culture	55
4.3.2.3 SDS-PAGE	56-57
4.3.2.4 LC-MS/MS of full length rLipL32, NrLipL32 and CrLipL32	57
4.4 Fragment comparison of LipL32 antigenic determinant	58-63
4.4.1 Fragment comparison of LipL32 fragments	58-61
4.4.2 ELISA of RCPAQAP samples using full length rLipL32	62-63
<b>CHAPTER 5 : DISCUSSION</b>	64-70
5.1 MAT positive and negative human serum samples collection	64
5.2 Determination of rLipL32 antigenic region using <i>in silico</i> methods	65
5.3 Production and analyze of the full length rLipL32 and its antigenic region	66-68
5.3.1 Production of full length rLipL32 and its antigenic fragments	66-67
5.3.2 Analyzing full length rLipL32 and its antigenic fragments	68
5.4 Fragment comparison of LipL32 antigenic fragments via ELISA	68-70
<b>CHAPTER 6 : CONCLUSION</b>	71-72
<b>REFERENCES</b>	73-97
<b>APPENDICES</b>	98-301

## LIST OF TABLES

	Pages
Table 2.1: Serogroup and some serovar of <i>Leptospira interrogans</i> sensu lato	8
Table 3.1: List of human serum samples used in this study	26-29
Table 3.2: List of oligonucleotide primers	30
Table 3.3: List of colony PCR reaction mix	33
Table 3.4: Table of 15% SDS PAGE resolving gel recipe	36
Table 3.5: Table of 5% SDS PAGE stacking gel recipe	37
Table 3.6: Table of SDS PAGE sample preparation	38
Table 4.1: 3 possible antigenic regions of full length LipL32 predicted by the Kyte & Doolittle hydrophathy scale	47
Table 4.2: 6 possible antigenic regions of full length LipL32 predicted by the recognition factor scale	48
Table 4.3: 8 possible antigenic regions of full length LipL32 predicted by the Kolaskar & Tangaokar propensity scale	50
Table 4.4: Yield of full length rLipL32, NrLipL32 and CrLipL32 from 1L of expressed culture	55
Table 4.5 ELISA result of 60 MAT positive human serum samples using the full length rLipL32, NrLipL32, CrLipL32 and intermediate LipL32	59
Table 4.6 ELISA result of 40 MAT negative human serum samples using the full length rLipL32, NrLipL32, CrLipL32 and intermediate LipL32	60
Table 4.7 Sensitivity and specificity of IgM and IgG when tested 60 MAT psotive and 40 MAT negative human serum samples using full length rLipL32, NrLipL32, CrLipL32 and intermediate LipL32	61
Table 4.8 ELISA result of RCPAQAP human serum samples when tested using full length rLipL32	62

## LIST OF FIGURES

	Pages
Figure 2.1: Biphase nature of Leptospirosis	11
Figure 2.2: <i>Leptospira interrogans</i> outer membrane protein	14
Figure 2.3: Epitope and paratope	17
Figure 3.1: ELISA template	40
Figure 3.2: ELISA template of LipL32 fragment comparison	42
Figure 3.3: ELISA template of RCPAQAP samples	44
Figure 4.1: Three possible antigenic regions of full length LipL32 predicted by the Kyte & Doolittle hydropathy scale	47
Figure 4.2: Six possible antigenic regions of full length LipL32 predicted by the recognition factor scale	48
Figure 4.3: Eighth possible antigenic regions of full length LipL32 predicted by the Kolaskar & Tangaokar propensity scale	49
Figure 4.4: Overlapping regions observed enabled full length LipL32 to be divided into 3 regions which were the NrLipL32 fragment (Ala <sub>21</sub> -Glu <sub>122</sub> ), the intermediate LipL32 fragment (Pro <sub>144</sub> -Glu <sub>171</sub> ) and the CrLipL32 fragment (Thr <sub>200</sub> -Ser <sub>261</sub> ).	51
Figure 4.5: Agarose gel analysis of colony PCR	52
Figure 4.6: Chromatogram showed presence of purified full length rLipL32 protein in fraction B9-B12 as indicated by the peak	53
Figure 4.7: SDS PAGE of recombinant LipL32 fraction collected	54
Figure 4.8: BSA concentration range from 0.125-8 mg/mL was used as standard curve for Bradford assay.	55
Figure 4.9: SDS PAGE of full length rLipL32	56
Figure 4.10: SDS PAGE of NrLipL23 and CrLipL32 fragments	57
Figure 4.11: Survey report of RCPA 2A and 2B samples	63
Figure 4.12: Survey report of RCPAQAP 4A and 4B samples	63

## LIST OF ABBREVIATIONS

CrLipL32 - Carboxyl terminal recombinant LipL32

Kg - kilogram

mg - Miligram

µg - Microgram

µL - Microlitre

1<sup>st</sup> - First

2<sup>nd</sup> - Second

Bp - Base pair

G - Gram

G - Gravity

Hr - Hour

Hrs - Hours

kDa - Kilodalton

Kg - Kilogram

L - Litre

Mb - Megabase

mL - Mililitre

NrLipL32 - Amino terminal recombinant LipL32

OD - Optical density

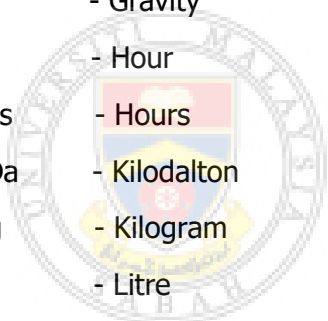
RCPA - Royal College of Pathologist of Australia

RE - Restriction enzyme

rLipL32 - Recombinant LipL32

Rpm - Rotation per minute

UV - Ultra violet



UMS  
UNIVERSITI MALAYSIA SABAH

## LIST OF SYMBOLS

% - percentage

°C - Degree Celcius

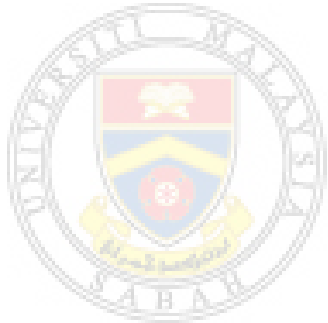


UMS  
UNIVERSITI MALAYSIA SABAH



## LIST OF APPENDICES

	Pages
Appendix A: Preparation of buffer solutions for electrophoresis	98-99
Appendix B: Preparation of buffer for ELISA	100-101
Appendix C: LC-MS/MS result of full length rLipL32, NrLipL32 and CrLipL32	102-120
Appendix D: ELISA graph result of MAT positive and negative samples when tested using full length rLipL32.	121-145
Appendix E: Comparison of CrLipL32, intermediate LipL32 and NrLipL32 fragments via ELISA.	146-295
Appendix F: ELISA tabulated result	296-301



UMS  
UNIVERSITI MALAYSIA SABAH

# CHAPTER 1

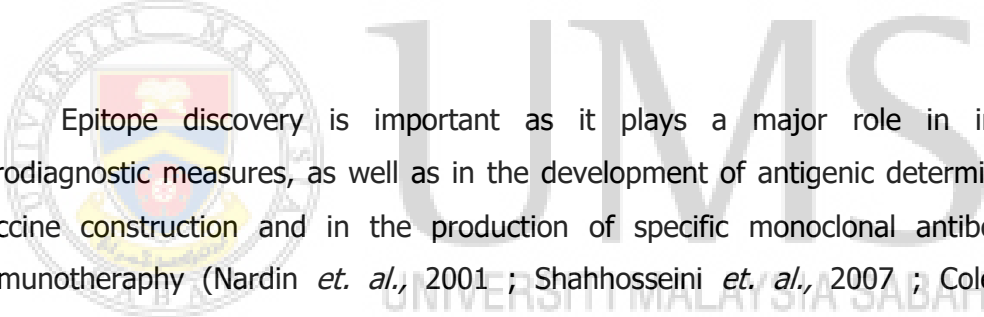
## INTRODUCTION

### 1.1 Background of study

Leptospirosis is a zoonotic disease caused by a pathogenic strain of the *Leptospira* bacterial spirochete, *L. interrogans* (Levett N., 2001). It is a predominant disease with incidence rates of 0.1-1/100 000 in temperate regions and >10/100 000 in tropical regions (WHO, 2013). Several countries have recently reported leptospirosis outbreaks such as in Sri Lanka, the Philippines and Austria (Agampodi *et. al.*, 2009 ; Koizumi *et. al.*, 2009 ; McCurry, 2009 ; Radl *et. al.*, 2011). In tropical countries such as Malaysia, leptospirosis is also one of the most prevalent zoonotic diseases with the highest number of fatalities reported in the year 2014 (92 fatalities) (Wahab, 2015). Both human and animals are found to be possible hosts for this disease. Leptospirosis is transmitted via ingesting contaminated food or drinks, or when exposed wounds or mucus membrane of the eye come into contact with contaminated water (Levitt N., 2001).

Diagnosis of leptospirosis suspected samples usually involve either culture of the microorganism on Ellinghausen-McCullough-Johnson-Harris media (EMJH) media, dark field microscopy (DFM), or by molecular diagnostics such as PCR and serology (Ahmad, 2005). In culture method, the isolated *Leptospira* will be culture on specialized media called EMJH media and observed under dark field microscopy (DFM). However this method is laborious, expensive, longer waiting time and it does not differentiate between pathogenic and non pathogenic *Leptospira* (Ahmad, 2005). Molecular method such as real time quantitative PCR, detect pathogenic *Leptospira lip32* gene has been reported before which provide rapid result (Levett *et. al.*, 2005). However it is

expensive to be used as routine application (Saengjaruk *et. al.*, 2002). The gold standard method which is MAT assay used the whole cell as antigen and able to determine pathogenic *Leptospira* serovar that is important in epidemiological study (Pappas *et. al.*, 1985). However this method is laborious, hazardous and delay in getting result. Rapid serological test such as ELISA utilize pathogenic *Leptospira* recombinant outer membrane protein (OMP) such as Lig A, Lig B, OMPL1, LipL21, LipL32 and LipL41 to detect *Leptospira* specific antibodies (Shang *et. al.*, 1996 ; Flannery *et. al.*, 2001 ; Raghavan *et. al.*, 2002 ; Okuda *et. al.*, 2005 ; Sankar *et. al.*, 2010 ; Joseph *et. al.*, 2012). This method is safer and cost effective than MAT assay, culture and PCR , however it cannot be used in epidemiological study. Of these, LipL32 is reported to be the most suitable as it is highly conserved and are abundant on the *Leptospira* OMP profile (Haake *et al.*, 2000 ; Flannery *et. al.*, 2001 ; Cullen *et al.*, 2002 ; Haake *et al.*, 2004 ; Tahiliani *et al.*, 2005 ; Levett 2007 ; Hoke *et al.*, 2008).



Epitope discovery is important as it plays a major role in improving serodiagnostic measures, as well as in the development of antigenic determinants for vaccine construction and in the production of specific monoclonal antibodies for immunotherapy (Nardin *et. al.*, 2001 ; Shahhosseini *et. al.*, 2007 ; Coleman *et. al.*, 2011 ; Gonzalez *et. al.*, 2014). Before bioinformatics algorithm were developed, epitope determination depended on empirical methods such as nuclear magnetic resonance (NMR), X-ray crystallography, electron microscopy (EM), ELISPOT and surface plasmon resonance (Reineke & Schutkowski, 2009). Currently various bioinformatic algorithms are developed to determine the antigenic determinants of an antigen based on different principles; such as hydrophilicity, hydrophobicity, solvent accessibility, secondary structure, antigenicity, flexibility and others (Chou and Fasman, 1974 ; Garnier, *et. al.*, 1978 ; Hoop & Woods, 1981 ; Kyte and Doolittle, 1982 ; Eisenberg, *et. al.*, 1984 ; Emini *et. al.*, 1985 ; Welling *et. al.*, 1985 ; Karplus and Schulz, 1985 ; Jameson and Wolf, 1988 ; Pellequer, *et. al.*, 1993).

Epitope determination of *Leptospira* OMP such as LigA, LipL21, LipL41 and OMPL1 have been reported previously using bioinformatics tools such as ANTIGENIC or MHCPreD which were then evaluated in ELISA for possible *Leptospira* specific antibodies detection (Xu *et. al.*, 2005 ; Wiwannitkit *et. al.*, 2007 ; Lin *et. al.*, 2008 ; Lin *et. al.*, 2010). Monoclonal antibodies derived from the LipL32 epitope has been reported to be able to neutralize leptospirosis infection in mouse models (Manewatch *et. al.*, 2013). However current methodologies on LipL32 epitopes investigations predominantly involve empirical studies such as immunoblotting, peptide array, truncated LipL32 recombinant protein based-immunoassay and phage consensus mimotope identification (Hauk *et. al.*, 2008 ; Lottersburg *et. al.*, 2009 ; Manewatch *et. al.*, 2013) while *in silico* investigations are still lacking (Lin *et. al.*, 2008 ; Lin *et. al.*, 2010). Importantly, *in silico* analysis have been mainly carried out using a single bioinformatic algorithm and there is currently no known study which involves the application of more than one algorithm for LipL32 epitope determination.

## **1.2 Problem statement**

Current methodology of LipL32 epitope determination has focus on experimental method rather than *in silico* method and there is no known research reported which involve application of more than one bioinformatic algorithm.

## **1.3 Hypothesis**

This study hypothesizes that the comparative analysis of 3 bioinformatic algorithms can identify an immunodominant LipL32 epitope of higher specificity and sensitivity for detection of *Leptospira* specific IgM and IgG antibodies in ELISA.

## 1.4 Objectives

The objectives of this study are :

1. To determine the possible antigenic determinants of LipL32 antigen via the utilization of 3 bioinformatic algorithms; the Kyte and Doolittle hydropathy scale, the recognition factor scale and the Kolaskar and Tangaokar propensity scale.
2. To determine fragments derived from the algorithm combinations for possible *Leptospira* specific antibodies with higher sensitivity and specificity for ELISA detection.



UMS  
UNIVERSITI MALAYSIA SABAH

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 History of Leptospirosis

Leptospirosis is a disease known by various names such as cane-cutter disease, 'Schlammfieber (mud fever)' and swine herd's disease. Ancient Chinese text has described it as 'rice field jaundice' while in Japan it has been known as 'autumn fever' or 'seven day fever' (Adler, 2015). The earliest recorded cases of leptospirosis dated back to 1886 by Adolph Weil, where he described the clinical features of severe Leptospirosis which are jaundice, splenomegaly, renal dysfunction, conjunctivitis and skin rashes, subsequently known as Weil's disease (Weil, 1886). It appeared to be infectious in nature and often associated with outdoor occupations in which a person came in contact with water. Epidemics were common among sewer workers, rice-field workers and coal miners (Landouzy, 1883). The first scientific demonstration of leptospire was conducted by Stimson in 1907 where he was able to observe the spirochetes using Levaditi silver staining technique in kidney tissue sections of a patient who suspected died of yellow fever. The patient was suffering from Weil's disease as spirochetes were observed only in the kidney but not in any other vital organs. The spirochetes were named *Leptospira interrogans* by Stimson, as it is known today, because it had hooked ends and resembled a question mark (Stimson, 1907). Isolation of the etiologic agent of Leptospirosis occurred almost simultaneously and independently in Japan and in Europe but Inada and Ido were the first ones to successfully isolate the pathogenic *Leptospira*, followed by two groups of German physicians that studied German soldiers afflicted with "French disease". (Uhlenhuth and Fromme, 1915 ; Hubener and Reiter, 1915 ; Inada *et. al.*, 1916).

## 2.2 *Leptospira interrogans* the etiological agent

Leptospirosis is a worldwide zoonotic disease that is caused by a pathogenic strain of leptospira called *Leptospira interrogans* (Levett, 2001). They belong to the order of Spirochaetales, family of Leptospiraceae, and the genus Leptospira. These spirochetes are both catalase and oxidase positive and grow in a simple media that contain vitamin B, ammonium salts and long chain fatty acids which they utilize as a sole carbon source (Faine *et. al.*, 1999). *L. interrogans* are long, thin, helical, highly motile spirochetes with two periplasmic flagella (endoflagella) and their cell envelope consist of a double layer structure in which their cytoplasmic membrane are closely associated with the peptidoglycan cell wall and overlaid by an outer membrane (Levett, 2001 ; Charon and Goldstein, 2002 ; Cullen *et. al.*, 2004). Their cell membrane share both properties of Gram-negative and Gram-positive bacteria but are found to be low in toxicity (Faine *et. al.*, 1999 ; Haake, 2000). Leptospires have a corkscrew-shape that differentiates them from other spirochetes by the presence of end hooks. They are about 0.1 µm in diameter and by 6-20 µm in length and require the use of dark field microscopy or phase contrast for observation (Faine *et al.*, 1999). They are also obligate aerobes with an optimum growth temperature ranging from 28°C to 30°C (Smilbert, 1977 ; Johnson & Faine., 1984; Haake, 2000). They are not resistant to drought or hypertonicity, however they are supported in alkalization to pH 7.8 (Mohammed *et al.*, 2011). The *Leptospira* genome consists of two circular chromosomes and complete genomic sequence of it has been established (Boursaux *et. al.*, 1998 ; Ren *et. al.*, 2003). Their genomes are larger than other spirochetes such as *Treponema* and *Borellia* which explains their ability to survive in varied environments such as animal host and freely in the harsh environment (Fraser *et. al.*, 1997 ; Fraser *et. al.*, 1998).

Serological classification of *Leptospira* consists of the pathogenic species, *Leptospira interrogans* and the environmental saprophytic strains *Leptospira biflexa* (Dikken & Kmety, 1978). Classification system depends on the antigenicity of their outer envelope (OMP) and they are further divided into various serovars after cross

absorption with available homologous antigen (Johnson & Faine., 1984 ; Kmety and Dikken, 1993). Antigenically related serovar are grouped into serogroups and currently *Leptospira interrogans* comprise 24 serogroups with over 200 serovars such as Canicola, Pomona, Hardjo, Grippotyphosa and others (Kmety and Dikken, 1993). Among the serovars encountered, Icterohaemorrhagiae has been reported to be associated with disease severity and seen in many countries (Storck *et al.*, 2010).

Genotypic classification based on DNA relatedness, reveal that *Leptospira* consist of several genome species such *L. alexanderi*, *L. alstonii*, *L. borgpetersenii*, *L. inadai*, *L. interrogans*, *L. fainei*, *L. kirschneri*, *L. licerasiae*, *L. noguchi*, *L. santarosai*, *L. terpstrae*, *L. weilii*, *L. wolffii*, *L. biflexa*, *L. meyeri*, *L. yanagawae*, *L. kmetyi*, *L. vanthielii*, and *L. wolbachii*, comprising both pathogenic and saprophytic species (Ramadass *et al.*, 1992). Genomic classification provides useful taxonomic data, however pathogenic and nonpathogenic serovar usually occur in the same species (Yasuda *et al.*, 1987; Brenner *et al.*, 1999). Thus, serological classification is used commonly, although this classification system does not have any taxonomic standing but they are useful for epidemiological purpose. Other than serological tests, conventional methods can also be used to differentiate between pathogenic and saprophytic strains. The ability of the pathogenic species to grow at low temperature in the presence of 8-azaguanine or formation of spherical cells in 1M NaCl distinguishes them from the saprophytic species (Kmety *et al.*, 1966)