THE STUDY OF RAPID DENGUE ANTIGEN DETECTING METHOD

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A report submitted in partial fulfilment of requirements for the degree of Bachelor in Electrical Engineering (Control, Instrumentation and Automation) with Honors

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SUPERVISOR'S DECLARATION

" I hereby declare that I have read through this "The Study of Rapid Dengue Antigen Detecting Method" and found that it has comply the partial fulfilment for awarding the degree of Bachelor of Electrical Engineering (Control, Instrumentation and Automation)"

Signature : .....

Supervisor's Name : DR. CHONG SHIN HORNG

Date : 20TH JUNE 2016

STUDENT'S DECLARATION

I declare that this report entitle "The Study of Rapid Dengue Antigen Detecting Method" is the result of my own research except as cited in the references. The report has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature: ……..

Author: GOH CHIA DI

Date: 20TH JUNE 2016

Gratitude to

My family

My FYP supervisor

My coursemates

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ABSTRACT

In the past decades, there are obvious technological improvement and development in diagnostic tests for infection pathogen. One of the most common infection pathogen is dengue virus. In this paper, a rapid dengue antigen detecting device is proposed with the combination of piezoelectric biosensor. The target antigen of this device is NS1 in human secretion. In the literature review part, a general introduction on dengue disease is discussed and the overview of the dengue diagnostic technologies is studied. Thus, a comparison is made in terms of time response and cost and summarized in the graph. Besides that, the current dengue diagnostic tests that used in clinic and hospital is also a part of literature review so that the device that is going to design is functional in real environment. The purpose of the piezoelectric biosensor is to detect the binding process of NS1 antigen on the conjugation part (mass changes) and convert to the electrical signal.

ABSTRAK

Dalam dekad yang lalu, terdapat peningkatan teknologi jelas dan pembangunan dalam ujian diagnostik untuk jangkitan patogen . Salah satu jangkitan patogen yang paling biasa adalah virus denggi. Dalam kertas ini, peranti yang pesat antigen denggi mengesan dicadangkan dengan kombinasi biosensor piezoelektrik. Antigen sasaran peranti ini adalah NS1 dalam rembesan manusia. Di bahagian kajian literatur, pengenalan umum mengenai penyakit denggi dibincangkan dan gambaran keseluruhan teknologi diagnostik denggi dikaji. Oleh itu , perbandingan telah dibuat dari segi tindak balas masa dan kos dan diringkaskan dalam graf. Selain itu, ujian diagnostik denggi semasa yang digunakan di klinik dan hospital juga merupakan sebahagian daripada kajian literatur supaya peranti yang akan direka bentuk berfungsi dalam persekitaran sebenar. Tujuan biosensor piezoelektrik adalah untuk mengesan proses pengikatan antigen NS1 di pihak conjugation (perubahan keberatan) dan menukar kepada isyarat elektrik.

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CHAPTER 1

INTRODUCTION

This chapter outlines the overall concept and idea of the project about the dengue disease, dengue virus and dengue diagnostic methods. Firstly, background and objectives of the final year project would be discussed. Then, problem statement, motivation and scope of the project would also be stated. The problem of time consuming process to diagnose dengue disease for patient and the evolution of technology for dengue disease. Therefore, rapid and miniaturized point-of-care (POC) test is recommended for diagnosing dengue in this research.

1.1 Project Background

Dengue is one of the killing infectious disease in both tropical and subtropical countries. Dengue become a major concern in tropical and subtropical climates cause it has caused approximately 2.5 billion people at risk and exceed 100 countries have widespread dengue virus transmission. The situation become worse during period of 2000-2004 (925896 cases), which the annual average was almost double if compared to the period of 1990-1999 (479848 cases) [1]. Dengue virus is defined as Flavivirus genus among the Flaviviridae family. It is a positive-sense single-stranded RNA virus which consists of a spherical shape of particle in 40-50nm diameter with a lipopolysaccharide envelope. Dengue fever is caused by four serotypes of dengue virus which are dengue virus 1 to 4 [2]. Based on the seroepidemiological studies, patient in primary infection is possessing a life-long immune response towards homologous dengue serotype. However, there is no protection for secondary infection patient with heterologous dengue serotype. This will lead to a major risk for both dengue hemorrhagic fever and dengue shock syndrome through an antibody-dependent enhancement [3]. There are several methods to

diagnose the dengue disease. In the early year of 1930, the virus culture and isolation techniques are the first laboratory test using to examine the dengue virus. Next, enzyme-linked immunosorbent assay (ELISA) is also known as serological assays has developed in 1980. The serological assays are widely used to identify dengue antibodies such as IgM and IgG. From the year of 2000 to 2010, the diagnostic test is focus on point-of-care (POC) diagnosis of dengue. The requirement target for ideal POC diagnostic test must comply with WHO's ASSURED [2].

1.2 Problem Statement

The dengue diseases such as dengue fever, dengue haemorrhagic fever and dengue shock syndrome (DF/DHF/DSS) are commonly found in tropical country. Among several methods, the technique of measuring the dengue specific antibodies in serum and other body fluids is adopted in this research. The relationship of kinetic response of IgG, IgM, and IgA antibodies during primary and secondary infection is important as a reference of diagnosing the dengue disease. Therefore, a low cost and rapid diagnostic device is developed to overcome limited setting resources in some of the tropical and sub-tropical country.

1.3 Motivation

Dengue disease has widespread in all regions especially tropical and sub-tropical climates country in recent decades. This disease has become global burden as there is no specific treatment such as vaccine to cure patient from this disease. The mortality rate is higher for the secondary dengue infection than primary dengue infection because secondary infection may lead to more severe form of disease such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Therefore, an analysis on the dengue diagnostic method is necessary for this research by implementing the hardware and software. The main concern behind this research is the early detection is crucial for patient to receive proper medical care so the fatality rates is lower.

1.4 Objectives

The main objectives of this project are:

1. To investigate and compare the existing dengue diagnostic device in terms of time response.
2. To propose an experimental procedure for the formation of MIP layer on QCM and the experimental setup.
3. To analyze existing result of MIP-QCM technology that used to detect the NS1 antigen.

1.5 Scope

In order to complete this project, the limitations are presented as follow:

i. The explanation and description on function of the chemicals that use to form the MIP layer on the QCM chip.
ii. The preparation of procedures for the experimental setup such as the formation of MIP layer process, materials and equipment that required to catty out the experiment.
iii. The method to analyze the performance of the MIP- QCM from the existing result by using the suitable equations.

CHAPTER 2

LITERATURE REVIEW

A general introduction of dengue disease is described in this chapter. After that, a detailed explanation on the evaluation of dengue diagnostic method and comparison of advantages, disadvantages, time response and cost between the methods. Then, the research on the dengue diagnostic test is done during the clinical visit. Next, the biosensor is introduced and comparison is made between QCM, SPR and EIS. Last, the lateral flow immunochromatographic test strip is described.

2.1 Dengue Disease and its immunological response of specific antigen and antibody

Dengue is an endemic viral / arboviral disease which consist of dengue fever (DF) and dengue haemorrhagic fever (DHF) and follow with dengue shock syndrome (DSS). This disease has become worldwide incidence as 2.5 billion people (40% of world's population) now live in transmission area especially tropical and subtropical area [2],[4]. The number of dengue cases from the year of 2009 until 2015 (2/11/2015) is presented in the Figure 2.1. Figure 2.1 shows a significantly increase of dengue cases and is treated as a serious disease in Malaysia [5]. The dengue disease spreads globally through vector mosquitos, Aedes aegypti in tropical and subtropical region ,Aedes albopictus in Western Pacific Region (South-East Asia, Africa, Australia, Europe and Americas) and Aedes polynesiensis in Pacific islands [2],[4].

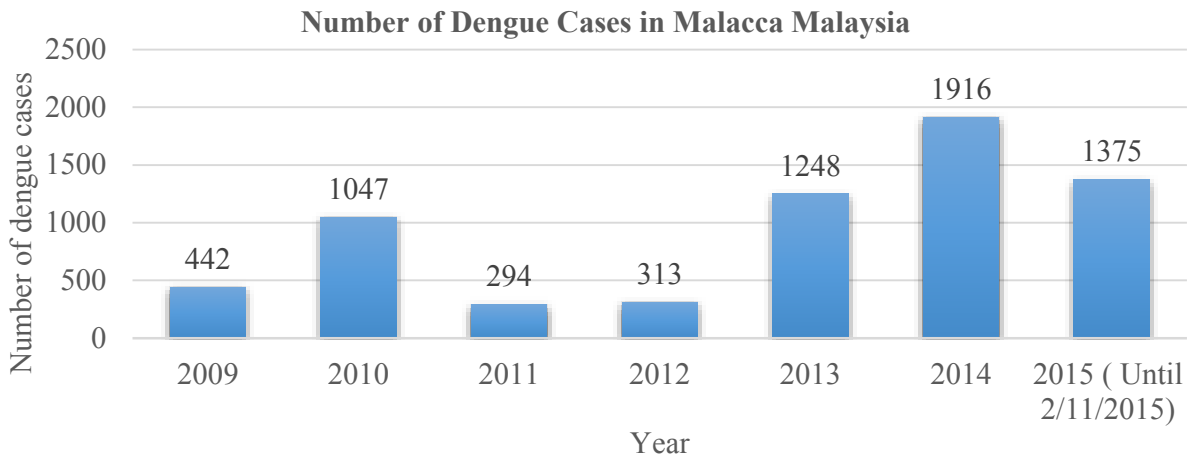

Figure 2.1: Number of dengue cases in Malacca Malaysia.

Next, the dengue disease is caused by a virus called Flavivirus genus which is a type of virus among the Flaviviridae family. The dengue virus is a single-stranded RNA virus with positive sense and consists of spherical shape particle in 40-50 nm diameter and with a lipopolysaccharide as outer layer of membrane. The genomic RNA has an essential feature that encodes 3 structural proteins which are core protein (C), membrane protein (M), envelop protein (E) and 7 nonsturctural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). There are 4 serotypes of dengue viruses such as dengue virus 1, dengue virus 2, dengue virus 3 and dengue virus 4 [2].

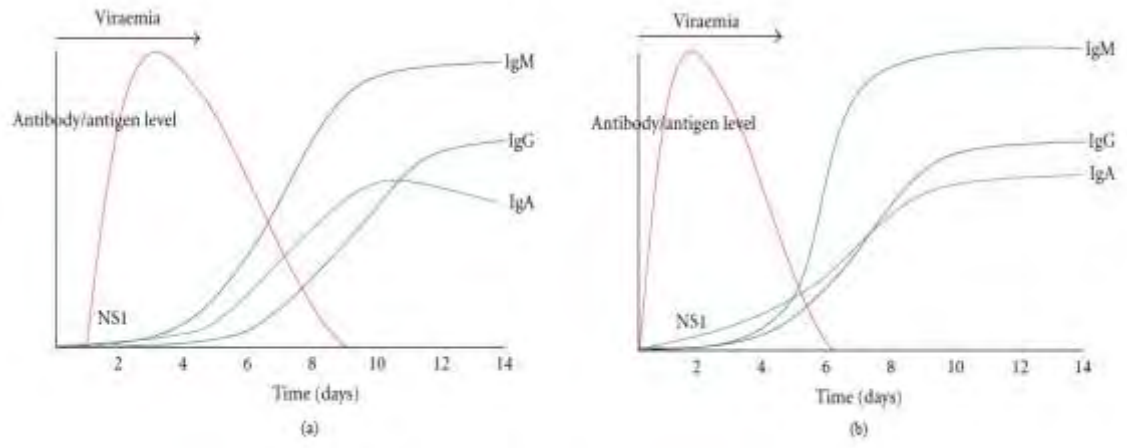

Figure 2.2: (a) Kinetics of dengue NS1 antigen and IgM, IgG, and IgA antibodies in primary dengue infection. (b) Kinetics of dengue NS1 antigen and IgM, IgG, and IgA antibodies in secondary dengue infection [1].

After that, the relationship of dengue diagnostic test is affected by the kinetic of dengue virus during primary and secondary dengue infection as show in Figure 2.2. In the initial dengue infection, a rapid replication of dengue virus in immune cell such as the liver and endothelial cell and indicate peak viraemia period as shown in Figure 2.2. At the same time, NS1 antigen can be found from 0 to 7 days following the onset symptom in the plasma, serum and circulating blood cell. Besides that, the difference between primary and secondary dengue infection in serological response can be referred to the Figure 2.2. Moreover, IgM antibodies develop follow by declination of viraemia phase from 3-5 days after the onset of infection while IgG antibodies only response after onset of IgM antibodies. For IgA antibodies, it can be detected between days 8 and 11 after onset of fever. The comparison of antibodies level also can be remarked to differentiate primary and secondary dengue infection especially IgG and IgM antibodies. This is the reason to be the common target by many rapid test kit. The level of IgM antibodies during secondary infection are lower than in primary infection while the level of IgG antibodies is higher in secondary infection if compared to the primary dengue infection [1], [2].

2.1.1 The overview of dengue diagnosis technology

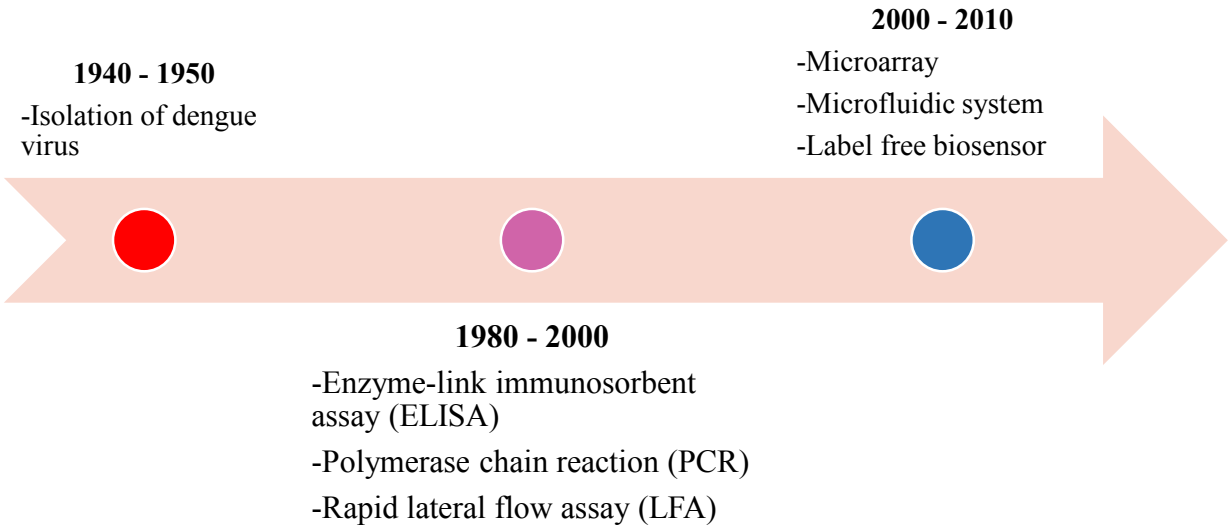

Figure 2.3: Basic timeline for dengue diagnostic technology.

Figure 2.3 shows the evaluation on the dengue diagnostic technology start from 1940 until 2010. The first method that used to detect the dengue virus by isolating dengue virus from liver, spleen, lymph nodes and other tissues and then carry out the immunofluorescent assay to identify the virus serotype using serotype-specific monoclonal antibodies. From 1980 to 2000, the new technology is introduced to detect dengue RNA, NS1 and IgM, IgG and IgA antibodies. Next, detection of dengue viral RNA by using reverse transcription – polymerase chain reaction (RT-PCR) amplification assay. This method has become new standard over the first method (isolation of dengue virus). For this test, the RNA is extracted from serum, plasma or cells then nested PCR technique is used to improve the sensitivity of detection. Although this technique is no less expensive and complex than virus isolation method, it is faster. A proper precaution and procedure is needed to prevent contamination that lead to false – positive result. The serological test is carried out by ELISA, rapid immunochromatographic strip (ICS) which is also called as lateral flow assay (LFA) to test the NS1 antigen, IgM, IgG and IgA antibodies. The advantages of these test is to differentiate between primary and secondary dengue infection if compared to the previous laboratory test. Due to the emerging technologies between years of 2000 to 2010, the rapid diagnosis of dengue disease can be replaced by 3 different types of label free biosensors

and 2 diminished platforms based on microarrays and microfluidic technologies. The 3 biosensors are quartz crystal microbalance (QCM), surface plasmon resonance (SPR) and electrochemical impedance spectroscopy (EIS). The quartz crystal microbalance is used to detect changes in resonance frequency when changes in mass provides electrically driven quartz crystal. QCM is a mass sensitive device based upon piezoelectric effect that function to detect the binding process between trace medical analytes and receptors on its surface. The SPR is an optical technique that detect the refractive index changes happening in the rapid vicinity of sensor surface upon analyte binding process. This is different with QCM that is affected by conformational changes (change in shape of macromolecule by environmental factor)in adsorbed species. Furthermore, the EIS biosensor rely on the electrochemical transduction such as direct measurement of current or voltage during analyte binding process [2], [6], [7]. The Table 2.1 shows the advantages, disadvantages and application of dengue diagnostic method [2].

Table 2.1: Conventional and emerging laboratory diagnostic methods for dengue infection [2].

Methods/ technologies	Advantages	Disadvantages	Major Application
Virus Detection (virus culture)	- Confirmation test - Specific - Identifies serotypes	-Requires high-level equipment, technical skills, manpower and time consuming (several days) - Expensive	- Detection of dengue pathogen
Nucleic acid amplification (RT-PCR)	- Confirmation tests - Fast (24-48 hours) - Sensitivity (80-90%) and specificity (>95%) - Serotype discriminating	- Easy sample contamination - High technological demands	- Dengue viral RNA detection
Serological assays (ELISA)	- Easy to perform - Fast (4-6hours) - Less expensive than virus isolation or RNA detection	-Requires equipment (microplate reader) - False positive due to cross-reactivity	- Dengue NS1, IgG or IgM detection
Point of care (Lateral Flow Test)	- Less time of analysis (15-20min) - Simple, user friendly and easy to perform - Require no extra equipment / supplies - Less expensive than virus isolation or RNA detection	- Mostly qualitative or semi-qualitative - False positive due to cross-reactivity - Less sensitivity and specificity than ELISA - Variable sensitivity and specificity among different commercial rapid tests	- Dengue NS1, IgG or IgM detection
Label free biosensor (QCM)	- Easy to use - Economy	- Prone to serum proteins interferences	- Detecting dengue viral protein (NS1,

	- Better sensitivity - Relatively simple technology in its production - Potential POC diagnosis	- Issue of robustness should be improved	E protein) - Viral genome
Label free biosensor (SPR)	- Real-time monitoring - Low sample consumption - High throughput - Remarkable sensitivity	- Bulky nature of the detection apparatus	- Detecting dengue viral antibody (IgM) - Viral protein (NS1)
Label free biosensor (EIS)	- Simple - Cost-effective - Quantitative - Ease of miniaturization - Provides time-dependent information	- Time-consuming technique - Moderate sensitivity	- Detecting dengue virus particles - Dengue oligonucleotide
Microarray	- Extremely high throughput - High sensitivity	- High running cost - Requires expensive equipment and high technical skills - Not suitable for routine and POC usage - Time consuming (several days)	- Detection and genetic analysis of dengue - To develop dengue prognostic biomarkers

			based on gene expression signature
Microfluidic platforms	- Miniaturization, increasing portability, disposability and suitable for POC usage - Low reagent cost, less waste, less required sample volumes - Faster analysis and response time - Better process control - Capability for parallel operation and multiplex capability - Minimized handling of hazardous material	- Novel technology and therefore not yet fully developed - Lack validation and standardization - Complex and somewhat expensive fabrication procedure	- Detecting dengue viral RNA, viral antibody (IgM and IgG)

2.1.2 Clinical Visit

There are two types of equipments that are using in Clinic UTeM to diagnose dengue disease such as full blood count method and serology with virology method. Table 2.2 shows the comparison of specification between these 2 methods. For dengue infection, the full blood count is the most common method that practice in hospital and clinic in Malaysia to check the level of white blood cell, platelets and the hematocrit (concentration) of blood. The normal limits of white blood cell in body is between 4000 μl to 12000 μl, the normal percentge range of hemotocrit of blood is between 35% to 55% and the platelet is between 150000 μl to 400000 μl. According to Dr. Shahaneen, a person who infected by dengue disease will has a low level of white blood cell and platelet while hematocrit of blood is high that is out of the stated normal range. Then, confirmation test is caried out by using combo test kit which use to detect NS1 antigen and IgG/IgM antibodies in body.