Lab-on-a-disk as a Potential Microfluidic Platform for Dengue NS1-ELISA

Nurul Aini Yusoff[1] *Student Member, IEEE*, Norhayati Soin[2], *Member, IEEE* and Fatimah Ibrahim[1], *Member, IEEE*
Department of Biomedical Engineering[1], Department of Electrical Engneering[2]
Faculty of Engineering, University of Malaya
50603, Kuala Lumpur, MALAYSIA
Email: aizas_05@yahoo.com

***Abstract*-Detection of non-structural protein 1 (NS1) of dengue virus can gives early diagnosis of dengue and shows high sensitivity. Current technique uses for the dengue NS1 detection is enzyme-linked-immusorbent assay (ELISA) on 96 microwell plate. However, the assay requires long incubation time about 90 minutes (for antigen–antibody interaction) and total assay time takes almost 2 hours and 30 minutes to complete. Therefore, a lab-on-a-disk with applied centrifugal force is proposed as a potential microfluidic platform to reduce the assay time by effectively mix and separate liquid in the ELISA assay. The advantages of the technique are having large specific volume, short diffusion length, minimum reagents consumption and simplify procedures. The lab-on-a-disk will exploit centrifugal and capillary forces to act as a passive valve to control the flow sequence of different solutions involved. Each steps of the ELISA process is carried out automatically by controlling the rotation speed of the disk. This paper will describe the lab-on-a-disk platform on its microfluidic principles, fabrication process, detection systems, biosensor applications, and the proposed model for dengue NS1-ELISA assay.**

***Keywords*—Lab-on-a-disk, dengue NS1-ELISA, centrifugal and capillary force.**

I. INTRODUCTION

Dengue fever is a viral disease transmitted by Aedes mosquito and the most important arboviral disease of human. Dengue usually happens in most tropical and subtropical area of the world including Malaysia. Some of the available laboratory diagnoses of dengue include virus amplification by polymerase chain reaction (PCR) process, detection of dengue specific antibodies i.e., IgM (Immunoglobulin M) or IgG (Immunoglobulin G) by enzyme-linked-immusorbent assay (ELISA), and detection of dengue non-structural protein 1 (NS1) antigen also by ELISA. The dengue NS1-ELISA has shown to give early diagnosis of dengue with high sensitivity [1]. Earlier diagnosis of dengue allows earlier implementation of supportive therapy and monitoring. Kumarasamy et al. 2006 [2] indicates that the commercial dengue NS1-ELISA may be superior to virus isolation and PCR for laboratory diagnosis of acute dengue infection based on single serum sample. In the NS1-ELISA kit, it uses microwell plate to test serum samples and takes almost 2 hours and 30 minutes to complete the whole procedures. The long time taken for the assay is due to extended time for incubation, high diffusion length of the well, uses lots of reagents, and process manually by the user. The extended time is required for incubation is attributed to inefficient mass transport of antigen/antibody from the solution to the well surface. However, a study by [3] found that immunoreaction of antigen and antibody is a rapid process.

Therefore, a novel technique is proposed to solve for the current problems by performing dengue NS1-ELISA on a microfluidic platform which is lab-on-a-disk or compact disk (CD). The small architecture of the CD can enhances the reaction efficiency by having large specific volume and short diffusion length. It can reduce the total assay time (little more than 1 hour), minimize reagents consumption, and simplify procedures [4]. The principal of the technique is it exploits centrifugal force and capillary force to properly mix the sample and reagents as well as to control the liquid flow. So, each steps of the ELISA process will be carried out automatically by controlling the rotation speed of the CD [4].

This technology has been developed successfully by Lai et al. 2004 [4] which applied ELISA on CD to detect rat antibody (Fig. 1). The CD has microfluidic structure that contained reservoirs, chambers, channels, and valves that function to fill and flow of liquid in the assay. The CD has a dimension of 12cm in diameter (similar to normal CD) and is made from polymethylmetacrylate, polydimethylsiloxane, or polycarbonate. In this paper, we will describe extensively about the lab-on-a-disk platform on its microfluidic principles, fabrication process, detection systems, biosensor applications and the proposed model for dengue NS1-ELISA assay.

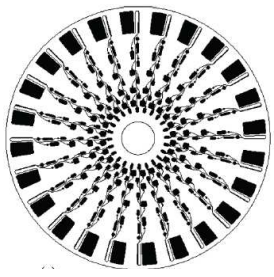

Figure 1: Example of CD designs for ELISA adopted from [4].

II. MICROFLUIDIC PRINCIPLES

The lab-on-a-disk combined several microfluidic functions for example centrifugal pumping, capillary valving and flow sequencing. The centrifugal and the capillary forces are used to control the flow sequence of

different solution in ELISA. Basic principles of these forces are briefly described below (adopted from [5]). In the lab-on-a-disk, centrifugal force provides the pumping pressure. The pumping force per unit area (P_c) due to the centrifugal force is given by:

$$\frac{dP_c}{dr} = \rho\omega^2 r \tag{1}$$

Where ρ= density of liquid, ω = angular velocity of the CD, r =distance of a liquid element from the center of the CD.

Integration of equation (1) from r=R1 to r=R2 (Fig. 2a) gives ΔP_c:

$$\Delta P_c = \rho\omega^2 (R_2 - R_1)\left(\frac{R_1 + R_2}{2}\right) = \rho\omega^2 \cdot \Delta R \cdot \overline{R} \tag{2}$$

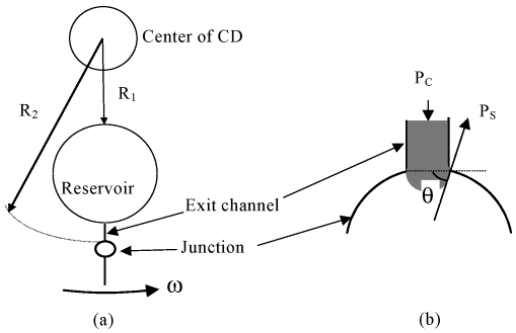

Figure 2: (a) Schematic illustration of fluid propulsion and (b) close-up view of the liquid front at the junction where the liquid is held by capillary force and produced capillary burst valve (adopted from [4]).

The capillary force produces a capillary burst valve that will ensure liquid from a reservoir is delivered to a measuring reservoir in a specified manner without releasing liquid from other reservoirs. This happened because the capillary force will stop the liquid flow at an extended opening (junction) of a microchannel (Fig. 2b). The liquid is released from the reservoir only when the applied ΔP_c is greater than ΔP_s. The performance of this passive valve is affected by the angular speed of rotation, fluid density, surface tension, and geometry and location of the channels and reservoirs [4]. The capillary force per unit area (P_s) due to interfacial tension is given by

$$\Delta P_s = \frac{C\gamma \sin\theta}{A} \tag{3}$$

Where γ = surface tension of the liquid, θ = contact angle, A = cross section area of capillary, C = the associated contact line length.

The burst frequency, f_b is the angular frequency at which ΔP_c is greater than ΔP_s. At this rotation speed, the liquid overcomes the pressure generated by the capillary force and flows to the capillary valve, releasing liquid from the reservoir. The burst frequency is calculated from equations 2 and 3 is given by:

$$f_b = \left(\frac{\gamma \sin\theta}{\pi^2 \rho \cdot \Delta R \cdot \overline{R} \cdot d_H}\right)^{\frac{1}{2}} \tag{4}$$

d_H= 4A/C, is the hydrodynamic diameter of the channel connected to the junction. By appropriate choosing channel dimensions and the junction size and location, precise flow sequencing can be obtained [6].

III. FABRICATION PROCESS

Lab-on-a-disk can be developed either by using conventional fabrication i.e. computer numerically controlled (CNC) machine or by using microfabrication technology i.e. photolithography. The choice of fabrication methods depend on the dimension of microstructure and quality of surface roughness. For large feature size (larger than 50μm), CNC machining can be used [7]. Besides, diamond-based micromilling and excimer laser based direct removal process can reduce the surface roughness to 1μm or less [8] but it is applicable for soft metal only (Al, Nickel, Copper). For smaller size (down to submicron), lithography (LIGA) -like method has to be used [9]. Lee et al. 2001 [7] have reviewed the fabrication process of mold inserts or master for CD microfluidic. The master has to be generated for repeatedly used in mass production process. In LIGA, different mold insert (plastic, glass, silicon, metal) for plastic molding (liquid resin, injection molding or hot embossing) can be created by bulk or surface micromachining [7].

A study by Lai et al. 2004 [4] has designed lab-on-a-disk patterns and was drawn using commercial AutoCAD software (AutoCAD 2000, AutoDesk, Inc). Channels and reservoirs (with depths ranging from 60 to 800 μm and widths ranging from 127 to 762μm) were generated on a poly (methyl methacrylate) plate (12 cm in diameter) by using a CNC machine.

Another study by Elizabeth et al. 2006 [10] had fabricated polydimetilsiloxane (PDMS) CD for the analysis of biomolecules using photolithography. The process started with 200μm thick of SU-8 photoresist was spin coated on a Si wafer. Then the fluidic patterns were transferred to the photoresist through a mask by UV irradiation. After immersion in the developer, the unexposed SU-8 photoresist was removed, revealing microfluidic pattern on the wafer. For CD fabrication, PDMS was cast onto the wafer and cured at 60^{o}C. The PDMS platform was then removed from the wafer and was cut into 12cm disk. The CD was covered with PC cover containing access port to match the design of PDMS for reagent loading. CNC machining can gives features containing channels and reservoirs with different depth but not in photolithography. However, features produced by photolithography are much better surface quality and accuracy.

IV. DETECTION SYSTEMS

There are two types of detection systems for lab-on-a-disk that have been developed which are portable and non-portable systems. One of the early types was developed by Madou et al. 2001 [5] which is a non-portable system and compose of assembly of bulk components (Fig. 3). In the system, the disk is mounted on a motor plate (up to 5000rpm) which is connected to an encoder to trigger a strobe for synchronized image. When the same position of CD passes under a charge-coupled device (CCD) camera, the strobe is triggered. So, only a fixed position of the CD is highlighted in each turn. The image of the CD is later sent to a computer for

data storage and analysis. The system has been used by Lai et al. 2004 [4] for ELISA rat antibody detection which captured the fluorescent intensity and analyzed the data using image analysis system.

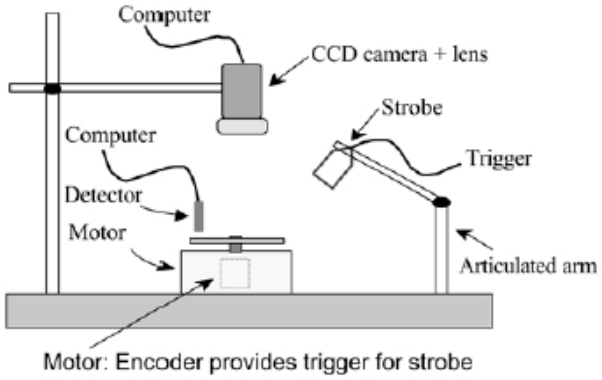

Figure 3: A non-portable CD microfluidic detection system (adopted from Madou 2001)

Another study by Morais et al. 2007 [11] was developed a compact, portable, and simple lab-on-a-disk detection system (Fig. 4). The application of this system is to sense low abundant molecules in a high density format with microgram per liter sensitivity. A set of servo systems (spindle and stepper motors) allowed disc rotation and laser scanning. The transmitted light through the disc is transformed by the photodiode into an analog electrical signal (rf signal). At the same time, the photosensor detects the trigger footprints and starting the data collection on the disc. The DAB (data acquisition board) brings the rf and trigger signals to the DAQ (data acquisition). The DAQ digitizes the analog signals and transfers them to the computer for processing. The detection system fully adapted from compact disc drive, improve and implement the former CD detection system to give high portability, versatile and sensitive. Most of the detection systems that have been developed for the lab-on-a-disk are similar to either of the first or the second design described here.

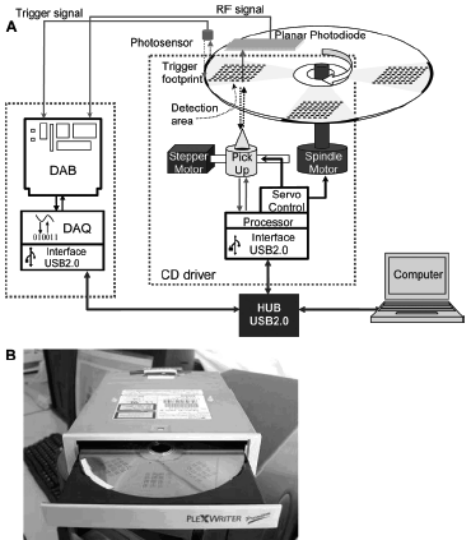

Figure 4: A portable CD microfluidic detection system (adopted from Morais et al. 2007)

V. BIOSENSOR APPLICATIONS

The lab-on-a-disk has been used in several applications of biosensor for rapid and automated assay. The design of the CD for its reservoirs, channels, and chambers varied and will depend on its applications. Nagai et al. 2007 was used CD microfluidic for the detection of secretory immunoglobulin A. It exploits heterogeneous enzyme immunoassay (EIA) technique by using a single bead immobilized with an antigen on the CD. The system was sensitive enough to detect a low level of secretory Immunoglobulin A.

Lai et al. 2004 [4] had applied lab-on-a-disk for the application of ELISA to detect antibody for rat IgG from a hybridoma cell culture. The rotation speed of the CD control the flow sequence of different solutions involved in ELISA.

Steigert et al. 2006 [13] developed a Bio-Disk which is a fully integrated colorimetric assay for the determination of the alcohol concentration in human whole blood. The result has shown on the CD platform within 150s and using just 500nL volume of blood. Meanwhile, Steinert et al. 2007 have demonstrated protein crystallization assay for proteinase K and catalase on CD.

Li et al. 2009 [15] used lab-on-a-disk for rapid DNA hybridization assay with nanoliter-volume of samples. The CD capable of generating the reciprocating flow of DNA samples within the microchannel. The device consists of PDMS CD slab with twelve DNA hybridization functional units and a glass substrate with immobilized DNA probe array. A reciprocating flow is produced with simple rotation-pause operation of the CD device.

VI. THE PROPOSED MODEL

The objective of the proposed model is to perform dengue NS1-ELISA on lab-on-a-disk for rapid dengue diagnosis. The structure of the disk will contain five steps of flow sequencing with five reservoirs (for reagents and sample loading), one detection chamber and a waste chamber (Fig. 5). There are also passive capillary valves which are the small circular shapes on top and bottom of the reservoirs to control the fluid flow. The reservoirs and chambers description is explained in Table 1.

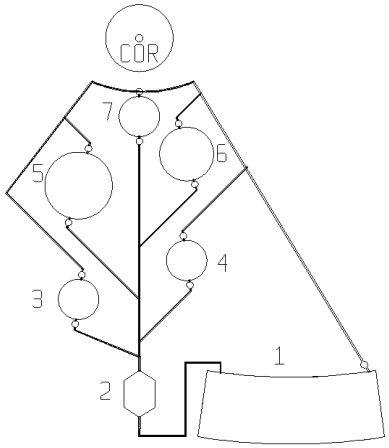

Figure 5: A single assay of five steps flow sequencing in the proposed model of lab-on-a-disk platform.

Table 1: Description of constituents for each reservoir / chamber of dengue NS1-ELISA assay on the CD.

No of reservoir	Description of constituent for each reservoir / chamber
1	Waste chamber
2	Detection chamber coated with Anti-NS1 Monoclonal Antibody (MAb)
3	Diluted serum
4	Enzyme solution (Horse radish peroxidase, HRP conjugated with Anti-NS1 MAb)
5	Washing solution (phosphate buffer saline with Tween 20)
6	Substrate solution (tetramethylbenzidine (TMB) and chromogen)
7	Stopping solution (sulfuric acid).

For the application of dengue NS1-ELISA assay on a CD, the following steps are proposed which is adapted from [4]. The detection chamber is first will coat with anti-NS1 MAb. Then, all reagents and sample will load into their reservoirs through access port on the CD cover. After that, rotation will be applied to the CD automatically by the detection system in increasing order to release the liquids sequentially to the detection chamber. The speed of rotation is in the range from 300 to 1500rpm [4]. Diluted serum will first release into the detection chamber to ensure the NS1 antigen and anti-NS1 MAb bind (10 min incubate). Later, the enzyme solution will release to develop a complex sandwich of (HRP-NS1MAb)-(NS1antigen)-(NS1MAb) bind (10 min incubate). Then, washing solution will release to remove non-specific binding (5minutes). Next, substrate will release to react with HRP enzyme in the complex to produce fluorescent color changes which shows the existence of dengue virus NS1 (5 min incubate). Then stopping solution will release to stop the reaction of the enzyme and substrate (2 min). Finally, the detection system will read the fluorescent intensity of the assay to give the result. The total assay time for this technique is around 35 minutes which is totally reduced from the conventional microwell plate (~2hrs and 30min). Besides, the depth of the detection chamber is only 400μm compare to microwell which is 8000 μm. So, the diffusion length will be shorter in the CD and gives faster liquid diffusion to the well surface as well as having large specific volume. Total volume of reagents and sample will use in the CD is only 75μl while in microwell is ~760μl, so very large reagents consumption is reduced in the CD. Therefore, we can have a potential diagnosis tool for dengue NS1 by applying the ELISA on a lab-on-a-disk platform.

In order to develop the lab-on-a-disk, the structural design of it needs to be studied properly to ensure the right dimension, shape, and position for the reservoirs, chambers and channels. Therefore, the lab-on-a disk is being modeled using Conventorware (Micro-electro-mechanical systems, MEMS software) to get an appropriate design. The photolithography fabrication process is modeled in the software to get the required microfluidic pattern of the CD with PDMS as the material. The developed 2D model of the CD is used to study extensively the fluid flow condition and capillary burst valve for each reservoir due to centrifugal pumping and capillary valving in order to control the flow sequencing. This will apply certain boundary condition to the design using finite element method. From the simulation results, we can develop the structural design of the CD for dengue NS1-ELISA assay. The CD will has three layers design which are cover layer (top), the structural layer with base (bottom) and an adhesive layer (in the middle). In a single CD, there will be four sets of a single assay that can be run in a time.

VII. CONCLUSION

The lab-on-a-disk has been reviewed as a potential platform to replace conventional microwell plate for dengue NS1 ELISA assay. It has the advantages of reducing total assay time, less reagent consumption, and automatic fluid control. Photolithography technique will give better surface roughness and accuracy of CD dimension compare to CNC machining. More simplified detection system similar to CD player will give high portability and can be used in point of care system. The lab-on-a-disk has been applied successfully as biosensor for laboratory assay. Therefore, performing dengue NS1-ELISA on lab-on-a-disk is a promising technique as fast diagnostic tool for dengue that can be model in MEMS technology. Modeling of the CD will show details analysis of the fluids flow inside each channels and reservoirs as well as giving an analytical result to be considered before developing the CD.

ACKNOWLEDGEMENT

This project was sponsored by University Malaysia Perlis and Ministry of Higher Education, Malaysia.

REFERENCES:

[1] H. Xu, B. Di, Y. Pan, L. Qiu, Y. Wang, and W. Hao et al., "Serotype 1-specific monoclonal antibody-based antigen capture immunoassay for detection of circulating nonstructural protein NS1: Implications for early diagnosis and serotyping of dengue virus infections," *J. Clin. Microbiol.* vol. 44, pp. 2872-2878, May 2006.

[2] V. Kumarasamy, A.H. Abdul Wahab, S.K. Chua, Z. Hassan, Y.K. Chem, M. Mohamad, and K.B Chua. "Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection," *J. Virol. Methods,* vol. 140, pp. 75-79, November 2006.

[3] J.S. Rossier and H.H. Girault, "Enzyme Linked Immunosorbent Assay on a Microchip with Electrochemical Detection," *Lab on a Chip*, vol. 1, pp. 153–157, 2001.

[4] S. Lai, S. Wang, J. Luo, J. Lee, S.T. Yang, M.J. Madou. "Design of a Compact Disk-like Microfluidic Platform for Enzyme-Linked Immunosorbent Assay," *Annal Chem* vol. 76, pp. 1832-1837, 2004.

[5] M.J. Madou, L. J. Lee, S. Daunert, S. Lai, and C.H. Shih. "Design and Fabrication of CD-like Microfluidic Platforms for Diagnostics: Microfluidic Functions," *Biomedical Microdevices* vol. 3, no. 3, pp. 245-254, 2001.

[6] L. Riegger, M. Grumann, and T. Nann et. al."Read-out concepts for multiplexed bead-based fluorescence immunoassays on centrifugal microfluidic platforms," *Sensors and Actuators* vol. 126, no. 2006, pp. 455-462, December 2005.

[7] L.J. Lee, M.J. Madou, K.W. Koelling, S. Daunert, and S. Lai et al. "Design and fabrication of CD-like microfluidic platforms for diagnostics: Polymer-Based Microfabrication," *Biomedical Microdevices*, vol.3, no. 4, pp. 339-351, 2001.

[8] M.A. Roberts, J.S. Rossier, P. Bercier, and H. Girault, "UV laser machined polymer substrates for the development of microdiagnostic systems," *Anal. Chem.,* vol. 69, pp. 2035–2042, 1997.

[9] M.J. Madou, *Fundamentals of microfabrication*, CRC Press, Boca Raton, 1997.

[10] A. Elizabeth, Moschou, A. D. Nicholson, G. Jia, J.V. Zoval, and M.J. Madou et al., "Integration of microcolumns and microfluidic fractionators on multitasking centrifugal microfluidic platform for the analysis of biomolecules," *Anal. Bioanal. Chem.,* vol. 385, pp. 596-605, May 2006.

[11] S. Morais, J. Carracosa, D. Mira, R. Puchades, and A. Maquieira. "Microimmunoanalysis on standard compact discs to determine low abundant compounds," *Anal. Chem.,* vol. 79, no. 20, pp. 7628-7635, 2007.

[12] H. Nagai, Y. Narita, M. Ohtaki, K. Saito, and M. Wakida, "A single bead analysis on disk shaped microfluidic," *The Japan Society for Analytical Chemistry* vol. 23, pp. 975-979, Aug 2007.

[13] J. Steigert, M. Grumann, T. Brenner, L. Riegger, J. Harter, R. Zengerle and J. Ducrée," Fully integrated whole blood testing by real-time absorption measurement on a centrifugal platform," *Lab on a Chip*, vol. 6, no. 8, pp. 1040–1044, May 2006.

[14] C.P. Steinert, J. Mueller-Dieckmann, M. Weiss, M. Roessle, R. Zengerle and P. Koltay,"Miniaturized and Highly Parallel Protein Crystallization on a Microfluidic Disc," *in Proceedings of 19th International Conference on Micro Electro Mechanical Systems (MEMS 2007), Kobe, Japan,* pp. 561–564, 2007.

[15] C. Li, X. Dong, J. Qin, and B. Lin, "Rapid nanoliter DNA hybridization based on reciprocating flow on a compact disk microfluidic device," *Analytica Chimica Acta* vol. 640, pp. 93-99, March 2009.