DEVELOPMENT OF LOW COST IMMUNOASSAYS IN AUTOMATED MICRODEVICES

Grando, V. V. N. ; Borba, J.C. ; Miranda, B. N. M. ; Carrilho, E.

vitoria.grando@usp.br;july0502@gmail.com;biamiranda@iqsc.usp.br;emanuel@iqsc.usp.br;

Abstract: *Enzyme-linked Immunosorbent Assay (ELISA) is an important method to diagnose plenty of diseases. Conventional ELISA requires multiple steps which enhance chances of contamination, and is a labor intensive technique. To overcome this problem there are many alternatives such as glass microfluidic devices, paper devices, among others that have emerged. In this project a cheap, simple, and fast microfluidic spin chip was developed. To study the design of the device, a simple and direct immune reaction related to Human-IgG and Anti-Human-IgG was chosen. The device was tested with water and PBS solutions. Both methods work very well with the design named ELISA 1.21, which opens new perspectives for joining microfluidic devices with immunoassays.*

Key-Words*: microdevices; immunoassay ; ELISA*

Introduction: ELISA is a specific combination reaction between Human-IgG and anti-IgG [1]. A colorimetric reaction enables antibody detection and its concentration based on the different intensities. However, it is an expensive method and presents more possibilities for contamination. For this reason the use of microdevices became an alternative to conventional ELISA. Some of the advantages related to the use of microdevices are a decrease in contamination by the use of communication channels and less sample manipulation. It doesn't rely on expensive equipment and the devices are made of cheap materials. Low cost technologies for diagnosing diseases are increasing the along with nano/micro technology and microfluidics. A lot of different materials are used for immunoassay tests such as paper [2], polyester [3], acrylic, glass, and PDMS [4], in order to decrease the test price. In this study, polyester was chosen due to its low cost, versatility and reusability.

Experimental: The structure of the device consists of three layers: base, intermediate, and top. The base and top layers consisted of a polyester film. The intermediate, however, was made of double-sided tape and polyester in alternated layers. The device design was drawn using AutoCAD 2016 software and cut using a CO_2 laser cutter (Kawai Laser 600 x 400 -Power 70 watts). Regarding the device construction, vents were made on the top side of the device to introduce samples and to enable solution movement among chambers. The chambers and valves were made in the middle layer. Finally, the base was a polyester film that was used as a base for antigen/antibody immobilization. In order to understand the flow parameters of each solution and optimize the device, water with colored dyes was used to track each solution and make sure its trajectory was in accordance with the desired trajectory, prior to immuno experiments. The polyester device was rotated using different rotational speeds that enabled sample movement between chambers and allowed the reaction to occur.

Results and discussion: Colored water and buffer solutions were added in chambers 1 through 5 (Figure 1). The device was rotated at different speeds--336 RPM, 442 RPM, and 580 RPM--and the solution moves with the same order as the numbers of the chambers. Siphon valves were used to increase the resistance. Consequently, solutions of chambers 3 and 4 take more time to pass through the central chamber. The reaction should happen in the central chamber where the Human-IgG would be immobilized. Valves and rotations were optimized and the design (Figure 1) works as expected for both solutions (water and water with colored dyes).

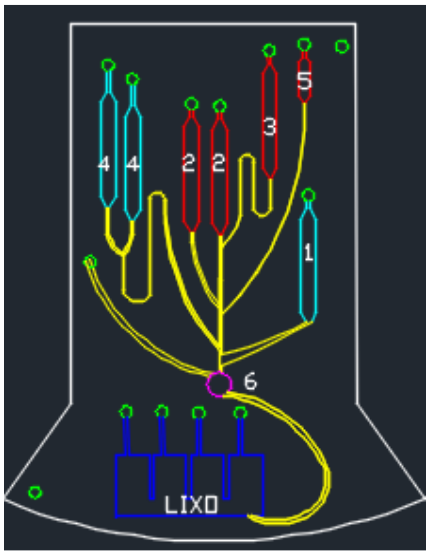

Figure 1: 1- BSA; 2 – PBS-t; 3 – Anti-IgG; 4 – PBS-t; 5 – TMB; 6 – central chamber.

When using ELISA solutions. The Human-IgG (1 mg/mL) should be immobilized before the central chamber is closed. The number 1 solution would be bovine serum albumin (BSA - 1 mg/mL) which moves to the central chamber at a rotation of 336 RPM (30 seconds and trash closed); the second solution would be PBS-t (tween - 0,075%) which is the washing solution and goes to the waste chamber with 336 RPM rotation (15 seconds and waste open). The third solution would be Anti-IgG (1:5000 in PBS-t), the fourth solution would be PBS-t. The fifth solution would be TMB (colorimetric solution) that will detect the horseradish peroxidase that is bonded to the Anti-Human-IgG and show that reaction happened. This part of the experiment was conducted in order to optimize ELISA spin chip.

Conclusion: The polyester double-sided tape microdevices present high potential for immunoassays. They highlight the fact that the microdevices should provide low sample and reagent consumption, as well as faster analysis when compared with conventional techniques. Both water and buffer solutions work very well with this design; therefore, the next step is to test the ELISA solutions. This device opens high possibilities for coupling microfluidic and biological assays for diagnostics.

References and acknowledgements:

[1] C.-M. Cheng, A. W. Martinez, J. Gong, C. R. Mace, S. T. Phillips, E. Carrilho, K. A. Mirica, and G. M. Whitesides, "Paper-Based ELISA," *Angew. Chemie*, vol. 122, no. 28, pp. 4881–4884, 2010.

[2] A. W. Martinez, S. T. Phillips, and G. M. Whitesides, "Three-dimensional microfluidic devices fabricated in layered paper and tape," *Proc. Natl. Acad. Sci.*, 2008.

[3] W. K. T. Coltro, S. M. Lunte, and E. Carrilho, "Comparison of the analytical performance of electrophoresis microchannels fabricated in PDMS, glass, and polyester-toner," *Electrophoresis*, vol. 29, no. 24, pp. 4928–4937, 2008.

[4] S. K. Sia and G. M. Whitesides, "Microfluidic devices fabricated in poly(dimethylsiloxane) for biological studies," *Electrophoresis*, vol. 24, no. 21, pp. 3563–3576, 2003.