紙型酵素免疫連結吸附法檢測血漿中乙型類澱粉蛋白早期診斷阿茲海默症

Toward Diagnosis of Alzheimer's Disease: Application of Paper-based ELISA to Detect β-amyloid Peptides in Plasma

[1]宋維瑄、[2]盧郁仁、[3]鄭兆珉

[1]林口長庚醫院 神經外科部 見習醫師 / 長庚大學 醫學院 大學部
[2]林口長庚醫院 神經外科部 主治醫師 / 長庚大學 醫學院 臨床專任教師
[3]清華大學 生物醫學工程研究所 專任教授

主要聯絡人
鄭兆珉 chaomin@mx.nthu.edu.tw

摘要：阿茲海默症，影響全球大約 4400 萬人口，是最常見的神經退化性疾病。目前阿茲海默症的診斷是基於臨床症狀，常用的診斷標準包含 NINCDS/ADRDA 和 DSM-IV。由於目前缺乏特異性高的檢測方法，阿茲海默症的診斷經常被忽略或延遲。在本實驗中，我們使用成本低的紙型酵素免疫連結吸附法 (Paper-based ELISA, P-ELISA) 檢測乙型類澱粉蛋白，此方法的優點在於實驗操作時間短大約只需要 85 分鐘，而且需要的檢體量少只需要 3 μL，此檢測方法被成功地用在緩衝系統和血清系統中偵測乙型類澱粉蛋白，靈敏度可達到 10 ng/mL。由以上結果，我們相信 P-ELISA 不論在學術上或臨床上對檢測阿茲海默症都會有莫大的助益。

Abstract : The single most common irreversible neurodegenerative disease, Alzheimer ‘s currently affects nearly 44 million people around the world. Existing diagnostic methods for Alzheimer’s disease, including NINCDS/ADRDA and DSM-IV, rely heavily on symptomology because no predictive or early-stage methods have been fully developed. Diagnosis of Alzheimer's disease is consequently missed or delayed in clinical practice. To remedy this situation, we have tested and propose further development of a specific paper-based ELISA process to determine β-amyloid peptide level in plasma as a means of cost-effective, early stage Alzheimer’s diagnosis. P-ELISA provides an excellent platform for diagnostic purposes in many regards, but most notably in its capacity to provide rapid results using low sample volumes. The protocol outlined herein requires only 3μL of sample per well and short operating time (only 85 minutes for the whole process). Our results indicate that monoclonal antibodies can successfully be used to detect β-amyloid peptide in both a buffer system and a plasma system, with a current detection limit of approximately 10 ng/mL in buffer. We truly believe that our P-ELISA-based approach for early detection of Alzheimer’s has the potential to be tremendously impactful for the population and may well prompt additional and expanded efforts in both academic and commercial communities.

關鍵詞：紙型酵素免疫連結吸附法、阿茲海默症、乙型類澱粉蛋白

Keywords : Paper-based ELISA, Alzheimer’s disease, β-amyloid peptide

Introduction

As the world's single most common irreversible neurodegenerative disease, Alzheimer's disease currently affects nearly 44 million people globally. [1] Therefore, it is helpful and necessary to develop a point-of-care diagnostic tool to provide the basis for timely clinical decisions. Here, we propose and provide supporting evidence for the further development of a cost-effective and rapid paper-based ELISA (P-ELISA) approach for early-stage, highly sensitive (pg/mL), and clinically relevant diagnosis of Alzheimer's disease. The discussed methodology has the potential for significant clinical impact and for additional expansion across a wide range of applications in both academic and commercial communities.

Application of paper-based ELISA to detect the β-amyloid peptides

Preparation of paper-based 96-well plates

The protocol of P-ELISA is basically similar to that of conventional ELISA, but plates constructed of paper with wax-printed wells are used rather than commercially available plastic microtiter plates.[2] We used a commercial wax printer (Phaser 8560, Xerox, Norwalk, CT, U.S.A.) to print the wax patterns onto filter paper (Whatman grade no. 1). Wax-printed plates were subsequently heated to melt the applied wax so that it penetrated from one side to the other, making complete hydrophobic wells on the paper device.

Paper-based ELISA for the detection of β-amyloid peptides in a buffer system

To detect the presence of β-amyloid peptides in 10-fold dilutions (from 1 μg /mL to 10 ng/mL) in the buffer system, a multiple-step procedure was developed to carry out P-ELISA[3], which is shown in Figure 2 and described as follows: (1) After rinsing, each test zone was immobilized with serially diluted β-amyloid [1-42] peptides for 20 min followed by 0.25% BSA blocking. (2) Three microliters of rabbit β-amyloid antibodies were placed into each test well, dried for 20 min at RT, and washed with phosphate-buffered saline containing 0.1% Tween-20 (PBST). (3) We placed three microliters of HRP-conjugated anti-rabbit IgG into each test well to act as a secondary antibody to detect the immobilized primary antibody and then we dried the plate for 10 min. (4) We washed each test with 5 μL PBST. (5) We spotted a mixture of 1.5 μL tetramethylbenzidine (TMB) and hydrogen peroxide (H2O2) onto the paper-based test zones to induce a measurable color change (from

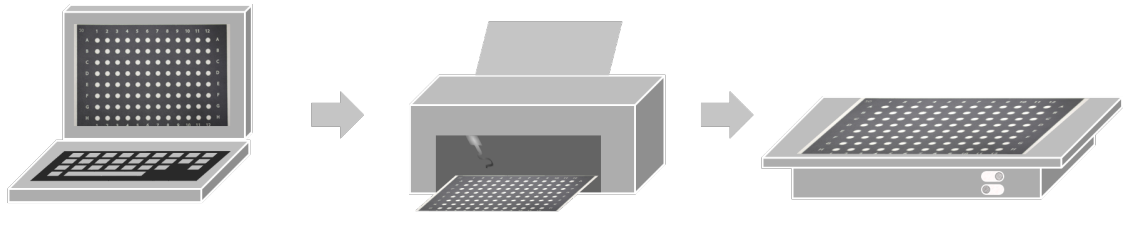

Figure 1. Schematic of the detailed procedure for making P-ELISA plates via wax printing: a) design 96-well plate pattern and layout design; b) wax printing onto filter paper (Whatman grade no. 1); c) wax melted by heating to generate hydrophobic barriers.

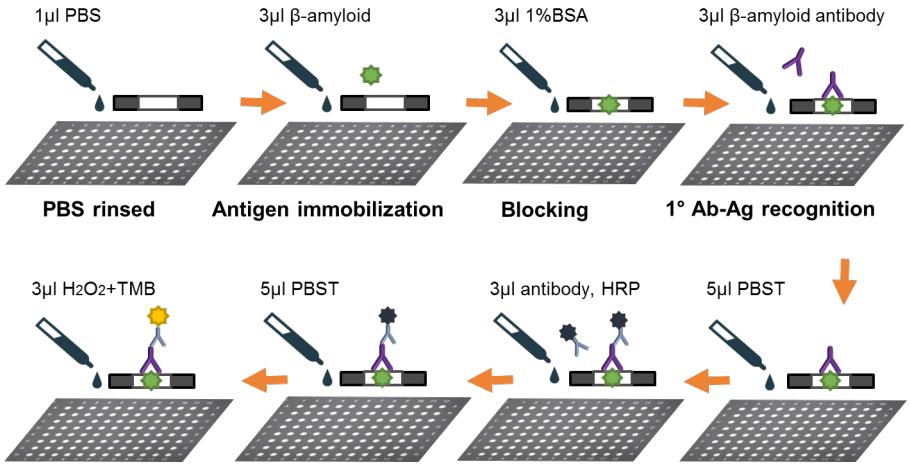

Figure 2. Schematic of the paper-based ELISA procedures for the detection of β-amyloid concentration.

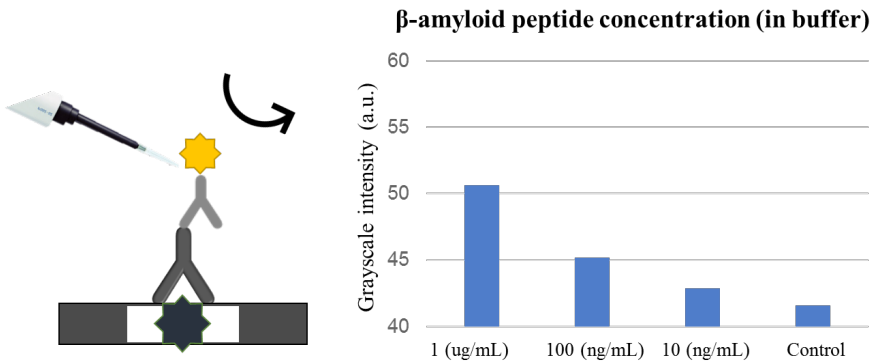

Figure 3 Graph of the greyscale intensity of the color developed in the test zones for the assays. The height of the bars represents the average of three independent measurements (N=3).

colorless to blue) and the plates were allowed to rest for 15 minutes. (6) We recorded color intensity for examination using a camera. (7) We analyzed color intensity to determine β-amyloid concentration. We determined the sensitivity of detection of rabbit IgG using an indirect P-ELISA to be 10 ng/mL. (Figure 3).

We first placed 3 μL of β-amyloid peptides onto each test zone after rinsing each test zone with 1 μL PBS. We spotted three microliters rabbit β-amyloid antibodies and then the same volume of HRP-conjugated secondary antibodies onto each test zone. In the final step, we used a 3 μL mixture of tetramethylbenzidine and hydrogen peroxide to produce a colorimetric response and recorded color for analysis to determine β-amyloid concentration.

Signal readout and statistical analysis

The color-based signal of our assay can be recorded using the following methods: 1) visual interpretation; 2) specific reader; 3) smartphone camera (Figure 4). In our previous study, we demonstrated a smartphone-based application that could record and analyze images and

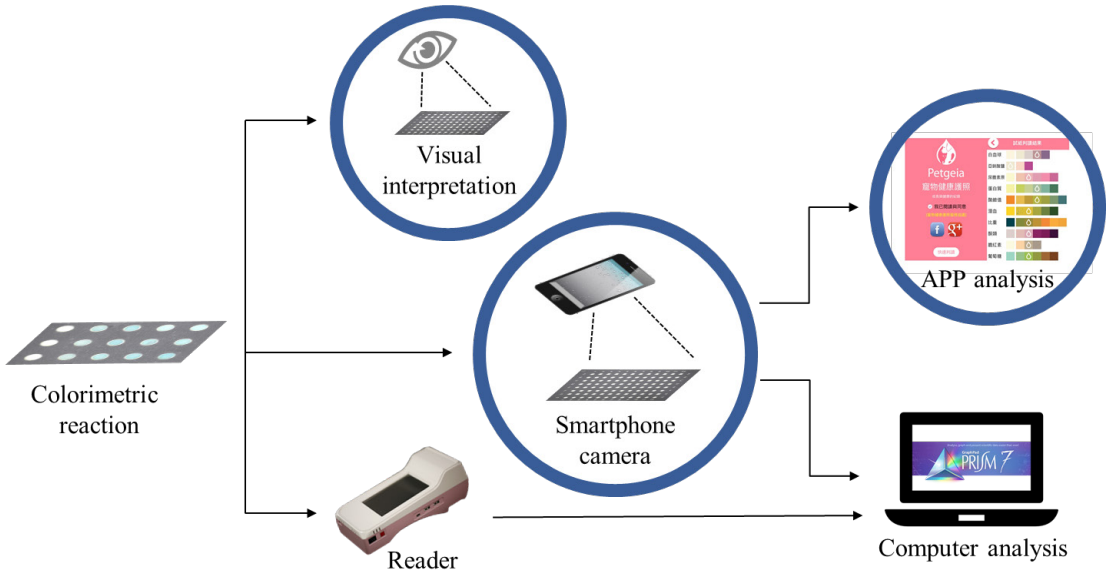

Figure 4. Color-based signal recording methods: 1) visual interpretation; 2) specific reader; 3) smartphone camera.

overcome recording issues including variances in ambient light and focal distance. In this study, P-ELISA results were defined as intensity of [experiment group] intensity of [blank zone]. We carried out all analyses using GraphPad Prism software.

Conclusion

This study is the first to establish a paper-based ELISA system as a tool for β-amyloid peptide detection, especially in human plasma. Because of our findings, we strongly believe that paper-based ELISA shows considerable promise for use as a credible tool for early diagnosis of Alzheimer's disease. However, the P-ELISA requires performance improvement including accuracy, precision, and long-term stability in order to be a commercially viable diagnostic device. Furthermore, the performance using actual clinical plasma samples needs to be evaluated. In conclusion, the P-ELISA system is so far a promising candidate for early diagnosis and a highly suitable tool for screening especially in small laboratories, and in developing countries where cost and convenience are more critical.

Acknowledgements

The study was supported by the Project 'CMRP-G3F0883', Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan.

References

[1] C. L. Masters, R. Bateman, K. Blennow, C. C. Rowe, R. A. Sperling, and J. L. Cummings, "Alzheimer's disease," (in eng), Nat Rev Dis Primers, Vol. 1, p. 15056, Oct 15 2015.

[2] S. J. Lo, S. C. Yang, D. J. Yao, J. H. Chen, W. C. Tu, and C. M. Cheng, "Molecular-level dengue fever diagnostic devices made out of paper," (in eng), Lab Chip, Vol. 13, no. 14, pp. 2686-92, Jul 21 2013.

[3] C. M. Cheng et al., "Paper-based ELISA," (in eng), Angew Chem Int Ed Engl, Vol. 49, no. 28, pp. 4771-4, Jun 28 2010.