行政院國家科學委員會補助專題研究計畫成果報告

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※自行研發之微毛細管電泳應用在糖化血紅素及肌動蛋白分析 ※

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計畫主持人：劉正民

共同主持人：何元順

計畫參與人員：

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

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□國際合作研究計畫國外研究報告書一份

執行單位：台北醫學大學

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中文摘要

本實驗包括糖化血紅素及肌動蛋白在毛細管電泳儀之試劑研發及毛細管電泳儀之硬體及軟體之組裝。在試劑的研發上發現用高濃度的Tris緩衝液用214nm波長之紫外線作偵測，可以達到良好的分離效果。血液樣本先將紅血球分離溶解後，用十倍的電泳緩衝液(300mM Boric acid + 100mM CAPS, pH = 11.0)稀釋後，注入毛細管電泳儀，在 10 kV 之電場下，糖化血紅素可清晰的與非糖化血紅素分開，然後用 System Gold 軟體將前者與後者之比率計算出。在肌動蛋白試劑研發上，由於純化之肌動蛋白及其抗體之售價高昂，本實驗室用兔子自行製備抗體。抗體經純化後與 Cy5 結合形成複合體(Ab-Cy5)，然後以不同濃度純化之 Troponin I 加入 Ab-Cy5，在電泳圖上形成復合體之新 peak，以線性回歸計算出相關性及相關係數，並以此作為標準曲線，作 Troponin I 之定量分析。

在硬體之研發方面，本實驗以 Xenon-Tunable 光源以 CV1 之軟體控制波長，經由 monochromator 通過一狹長(2mm long, 0.1mm wide)之 pinhole，恰巧與毛細管偵測 window 吻合，偵測部分分別以 1L 1400 Photometer 及 PMT 檢測其靈敏度，目前的結果發現兩者均可偵測到訊號，其優缺點正在評估中。在螢光標籤雷射激發硬體之裝置，由於經費之限制，與工業技術研究所合作，目前已裝配完成並開始作檢體之測試。

關鍵詞：簡易型毛細管電泳，糖化血紅素，肌動蛋白

英文摘要

The goal of this project include; 1) the development of test reagents for Hb A1c and troponin I in capillary electrophoresis and 2) assembly a simple and low cost capillary electrophoresis. In the first part of experiment is to develop and optimize the running buffer and running conditions. High ionic strength of Tris based buffer and boric acid buffer were used for Hb A1c analysis. The percent of Hb A1c from total Hb were calculated by integrating the peak area by System Gold (Beckman Instruments) and the ratio was calculated by Excel. Troponin antibody was developed by immunization of rabbit, the serum was collected and purified and the antibody titer was evaluated by ELISA. The conjugation of Cy5 to antibody was followed by the package insert of commercial product. The conjugated Ab-Cy5 was separated from the free Cy5 by PD-10 column. The conjugate was subject for injection to capillary electrophoresis. The fluorescence was detected by laser-induced-fluorecence with He/Ne laser (635 nm excitation light and the emission light was collected by 675 nm band pass filter). The fluorescence intensity and migration time were showed in the electropherograms. The preliminary results showed that the peak area of Ab-Cy5-Troponin is correspondence with the amount of troponin added. The correlation standard curve was generated and the amount of troponin can be calculated from the curve. The second part of experiments is to assemble a simple and

low cost capillary electrophoresis. The experiment was conducted by using xenon-tunable light source and the collimated light was controlled by software and monochromator. The light was focused on a narrow slit with 2 mm long and 0.1 mm wide slit which just behind a window part of capillary. Two types of detector were used, one is photodiode (IL 1400 Photometer) and the other is PMT. The sensitivity has been evaluated currently.

Keywords: capillary electrophoresis instrument, glycated hemoglobin (Hb A1c), troponin

計畫緣由與目的:

Capillary electrophoresis is a new analytical technique with fast analysis and high accuracy for either small or large molecules. The technique has been applied to clinical diagnosis because fast separation, high resolution and accuracy. However, the technique has not be used widely because the high cost and low throughput. Current commercial instruments are designed for research purpose and they are hard to adapt to clinic laboratory. The purpose of this study is to desigh and assemble a simple and low cost instrument for clinical usage. Hb A1c and troponin were selected as the pilot target because the bigger market potential and higher degree of emergency. Hb A1c is the standard assay for diabetes diagnosis and follow-up after medical treatment, and troponin is the best marker for AMI diagnosis. In this feasibility and viability study, we provide a new technique and instrument for clinical diagnosis.

結果與討論:

From the results of this study, two running buffer for analysis of Hb A1c were developed. One is 300 mM boric acid with pH 11 (adjusted by using 1 N NaOH). The other is 677 mM tris buffer with pH 8.3. The migration time is 20 min and 18 min respectively. For troponin assay reagents, first of all the polyclonal antibody was produced from rabbit. The serum of troponin immunized rabbit was collected and the IgG was purified by ammonium sulfate precipitation method. After dialyzed with PBS, the purified antibody was tittered by ELISA. Cy5 (cyanine) was purchased from Amersham Pharmacia biotech and then diluted with bicarbonate bufffer to a concentration of 1 mg/ml and incubated with purified AB in the ratio of 1/10. The mixture then checked by capillary electrophoresis equipped with Ne/He laser module to make sure the conjugate has formed. The unconjugated Cy5 was removed by PD-10 or P-30 polyacrylamide gel column. The conjugate (Ab-Cy5) was collected and aliquot into 50 μL/ vial and stored in –20°C freezer until be used. The purified troponin (Sigma) was diluted by bicarbonate buffer from 1 μg/ml to 8 μg/ml, the complex (Ab-Cy5-troponin) formed which indicated in the resultant

electropherogram. The correlation between the peak areas of the complex/ internal standard peak (from free Cy5) vs. the amount of purified troponin was calculated by linear regression analysis.

In the homemade CE experiment, a mini-type of high voltage power supply (Spellman, MM 15-2.5 W) was substituted for the conventional one. The maximal output power is 15 kV, 170 mAmp which is fit for general analysis. The light source was used xenon -tunable light source and pass a monochromator (CVI laser Corporation, type:CERMAX LX175F). The wavelength range from 200 nm to 1100 nm and is controlled by computer. The focus length is 110 mm and the light pass a slit (0.1 m x 2 mm) to the detection window of the capillary. The detector was used in this study was an IL 1400 A photodiode (International light Inc., SED 033/NS 633). The signal was analyzed by computer software. This self-assembly capillary is much simpler than the conventional instrument and lower cost. In the preliminary test, the signal can be detected in the dark field. Currently, we try to put the detection device into a black box to solve the problem.

計畫成果自評:

From the results of this experiment, the running conditions were generated for both Hb A1c and troponin. The antibody for troponin was produced and purified which cut down the cost of assay. The conjugation process is not very stable so far, which may need to manufacture a big lot and to make the assay become more consistent. The results for hardware assembly found that to cut down the cost and maintain the assay quality is feasible. From this prototype CE, it also easy to fit to microfluidic microchip detection in our future studies.

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