以毛細管電泳之雷射誘發螢光分析唾液中分析型免疫球蛋白 A 之含量

Analysis of secretory IgA in human saliva by laser induced fluorescence capillary electrophoresis

中文摘要

許多疾病引起的變化可從唾液中偵測出來,唾液的檢驗日益受到重視,因爲檢體來源取得方便且爲非侵入性,用其來發展相關的疾病檢驗或當作生理健康指標有其特殊意義。人體唾液中之分泌型免疫球蛋白 A,secretary IgA (sIgA),爲身體抵抗外來物侵襲的第一道防線,病毒或細菌的感染會造成唾液中的(sIgA)增加,而壓力累積或免疫衰竭時 sIgA 則減少,唾液中 sIgA 多寡,可反應出個人免疫力之強弱,也可視爲一種健康指標。毛細管電泳是一種具有潛力的分析工具,它具備快速、簡單、解析度高並能自動化的優點。本文研究的目的是利用毛細管電泳儀對唾液中之 sIgA 含量，尋找出最佳化的分析條件，及建立定量分析的方法。由於唾液中之 sIgA 含量較血清爲低，以 UV/vis 作偵測其靈敏度不夠，所以本實驗採用雷射激發螢光法(Laser induced fluorescence ,LIF)來提高偵測靈敏度,以 Cy5 爲螢光劑,其最大激發波長在 649nm，散射光波長在 670nm。毛細管電泳法採 (Capillary zone electro- phoresis,CZE)並以競爭性毛細管免疫測定法 (competitive capillary immunoassay) 和非競爭性免疫測定法(direct immunoassay)分析受試者於考試前後唾液中的 sIgA 含量之變化。其結果顯示前者穩定不如後者,以抗原抗體反應形成複合體之量與抗原之量成正比,以純化之 sIgA 建立標準曲線,由此一曲線從被測者之複合體在電泳圖中形成之波峰及內在標準(internal standard)之波峰可以計算出 sIgA 之含量。

英文摘要

Saliva is extremely sensitive to changes in oral and systemic diseases. As a new role for clinical tool, saliva offers many advantages over serum, saliva is easy to collect, painless and non invasive. It can be used as an indicator on the status of individual immunity. Secretory immunoglobulin A (sIgA) is the most abundant immunoglobulin in saliva and plays an important role in mucosal immunity. sIgA measurement in saliva as an index of mucosal immunity, has repeatedly been shown sensitive to physiological variables. For instance increase level comes after bacterial or viral infections, contrary reduction level can be observed while under stressful circumstances.

Capillary electrophoresis (CE) is a new analytical technique for assessment of different variety of body fluids, as it requ.ires only a small volume and provides a

rapid analysis with high efficient separation. In addition the instrument is easy to be automated. This study aims to establish an optimal condition for CE analyzed trace amount of sIgA in saliva. Laser induced fluorescence (LIF) conducted in capillary electrophoresis improvement of its sensitivity, both of the competitive (CI-CE) and the non competitive (direct) immunoassays (NCI-CE). The fluorescence Cy5 on the maximum absorption at 649 nm and the maximum emission efficiency at 670 nm was used for either sIgA or anti-sIgA labeling. The electrophoresis was conducted under 15kV in borate buffer 150 mM with CHAPS, pH8.5. The method is based on the different mobility of the free and bound tracer (fluorescently labeled compound). The amounts of sIgA in graduate student''s saliva were assessed during the period under stress or stress free. The results indicate that the NCI-CE is more stable and reproducible than the CI-CE for sIgA quantitative analysis.etes.