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Editorial

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Commentary on "Comparison of two different HPLC methods and ELISA method for measurement of serum neopterin"

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Commentary

Neopterin measurement is performed by various methods such as high performance liquid chromatography (HPLC), radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), electrophoresis, thin layer chromatography, paper chromatography. Today, HPLC is considered to be the gold standard method all over the world.

Environmental Health is that the branch of public health that focuses on the interrelationships between people and their environment, promotes human health and well-being, and fosters healthy and safe communities. As a fundamental component of a comprehensive public health system, environmental health works to advance policies and programs to reduce chemical and other environmental exposures in air, water, soil and food to protect residents and provide communities with healthier environments. In this study, we compared the serum neopterin levels with two HPLC method and commercial neopterin ELISA kit. HPLC method using potassium phosphate buffer (pH: 6,4) as mobile phase and trichloroacetic acid for deproteinization was named as "method 1"; and the method which we have recently developed using water/acetonitrile (99/1, v:v) as a mobile phase and acetonitrile for deproteinization, was named as "method 2". Neopterin retention time was 6,4 min. for method 1 and 2,4 min. for method. The linearity of method 1 and method 2 was quite good (r2: 0.987 and r2: 0.998, respectively). Repeatability of method 2 was higher than method 1 and commercial kit. In conclusion, our developed HPLC method has better analytical performance and shorter analysis time and easier to apply with respect to the HPLC method.

Neopterin is a pyrazino-pyrimidine compound belonging to the pteridine class, which is only produced by living cells [1]. Neopterin is produced from guanosine triphosphate by stimulated macrophages under the effect of lymphocyte-originated gamma interferon. Neopterin is an important marker which plays a key role in the interaction of monocyte/macrophage activation [2]. Neopterin was demonstrated to be a sensitive marker of cell-mediated immune reactions; therefore, the identification of neopterin levels in various body fluids has diagnostic significance in numerous diseases including the diseases of T-lymphocytes and macrophages.

ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying soluble substances such as peptides, proteins, antibodies, and hormones. Other names, such as enzyme immunoassay (EIA), are also used to describe the same technology. In an ELISA, the antigen (target macromolecule) is immobilized on a solid surface (microplate) and then complexed with an antibody that is linked to a reporter enzyme. Detection is accomplished by measuring the activity of the reporter enzyme via incubation with the appropriate substrate to produce a measurable product. The most crucial element of an ELISA is a highly specific antibody-antigen interaction.

Comparison of methods

For method comparison, 100 serum samples in different neopterin concentrations (2.79 - 54.2 nmol/L) were used. Neopterin levels of the serum samples were determined by three methods.

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KruskalWallis one-way ANOVA t-test for paired samples was used for the comparison of serum neopterin levels measured by HPLC and ELISA. The statistical package SPSS for Windows, version 15.0 (Chicago, IL, USA), Microsoft Excel and EP Evaluator Release 8 (Canada, USA) was used for statistical procedures.

In conclusion, we develop a new fast and easy method for serum neopterin measurement.

The use of acetonitrile rather than acidic conditions to precipitate and remove serum proteins provided a more accurate neopterin result in samples. Water/acetonitrile mobile phase provided shorter analyze time and better chromatogram according to peak shape and baseline noise. Also, analytical method validation performance of our new method was better than the other methods.