Performances of Point-of-care Testing Systems for HDL Cholesterol

Woo-Churl Chae, Jeoung-Hwan Cho, and Se-Hwan Paek
Lab. of Biosensor System Engineering, Korea University
TEL: +82-2-3290-3438, FAX: +82-2-927-2797

Abstract

Plasma lipoproteins transporting cholesterol through blood vessels are divided into three major classes, VLDL, LDL, and HDL. The ratio of HDL cholesterol over the total can be used as an indicator for prognosis of coronary artery diseases. In this study, we have developed two analytical systems for %HDL cholesterol with different flow modes toward gravity and analyzed them for their characteristics and performances.

Introduction

Cholesterol is an essential material in the human body, as the precursor of steroid hormone and a constituent of cell membranes. The lipophilic substance is transported between peripheral tissues and the liver by plasma lipoproteins. Three major classes of the lipoproteins, classified according to mainly their densities, are very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).[1] It has been reported that decreased HDL cholesterol (HDL-C) as well as increased LDL cholesterol (LDL-C) are independent factors causing coronary heart disease (CHD).[2] Various methods for the quantitative measurement of LDL-C or HDL-C have been reported. Most methods use prior isolation of a single lipoprotein class before measurement of the cholesterol level. However, these procedures do not require only laboratory instruments but also discrete accomplishment in regard to the subsequent quantification of cholesterol. To avoid such limitations, we have devised two membrane systems for HDL-C by stacking membrane pads with each unique function required for the cholesterol assay.[3] The systems employed basically a flow-through mode of sample to generate a uniform color signal on the surface of pads and they were constructed in two different formats: gravity-dependent system (i.e., flow along the gravity) and gravity-independent system

(i.e., flow against the gravity). The gravity dependency may affect particularly the flow rate of sample flow through the membrane pores and the uniformity of color signal. Therefore, we have analyzed them for characteristics and performances according to the direction of sample flow.

Material and Method

Construction of gravity-dependent system (GDS). The gravity-dependent system for HDL-C consisted of three major components: pad for selective precipitation for LDL and VLDL, pad for filtering red blood cells and lipoprotein precipitates, and pad for signal generation resulting from consecutive reactions of three enzymes, cholesterol esterase (CE), cholesterol oxidase(CO), and horseradish peroxidase (HRP). Each pad were completely superimposed in the order from the top.

Construction of gravity-independent system (GIS). The gravity-independent system was composed of five functional pads for: sample absorption, filtration of red blood cells, separation of precipitated LDL and VLDL, enzymatic decomposition of lipoproteins and cholesterol, signal generation using HRP in the presence of tetramethylbenzidine (TMB). These pads were effectively located from the bottom of the system.

Analysis. Using the two systems constructed, sample was absorbed from the precipitation pad for GDS and sample absorption pad for GIS. Along the migration path of sample by the capillary action, the analyte reacted with each reagent present in each pad consecutively. The separation of LDL and VLDL was accomplished based on chemical precipitation using dextran sulfate as polyanion and $MgCl_2$ as divalent cation.[4] The precipitant, selectively reacting with LDL and VLDL, produced LDL and VLDL particles in large sizes. After removing the particles, only HDL cholesterol was decomposed and hydrogen peroxide was consequently produced. This product reacted with enzyme HRP in the presence of TMB. As a result, TMB was oxidized, a blue-green color was generated as signal proportional to the concentration of HDL cholesterol, and the color

was finally quantified by an optical sensor.

Result and Discussion

Gravity-dependent system. In GDS, because the sample was migrated by gravity as well as the capillary action, the flow rate was very high and the time for reacting with reagents present in the pads was relatively short. This characteristics resulted in a short analysis time and low capacity in separating LDL and VLDL comparing to those of GIS. However, GIS was more suitable for generating a uniform color signal on the pad owing to the rapid flow. Because the uniformity of signal can increase the reproducibility of analysis, GDS had a lower variation of data than that from GIS.

Gravity-independent system. In GIS, since the sample was delivered only by the capillary action against gravity, the flow rate of sample was low and there was a time enough for enzyme and chemical reactions. Thus, the capacity of LDL and VLDL separation was high and regent concentration required for the assay was relatively low comparing to that in GDS. To compensate for the color uniformity, 10 mM phosphate buffer, pH 6.0, was used as medium and this consequently reduced the sample volume required for assay. This can provide an additional advantage by diminishing the effect of interference factors contained in blood such as hematocrit, ascorbic acid, uric acid, and bilirubin. Using such conditions, multiple analytes can be simultaneously measured only by a single finger prick.

Performance comparison. In the both systems, the intensities of the color signals were approximately constant 90 sec after the sample application. The assay time was variable according to the concentrations of reagents used. Relative positions of dose-response curves obtained from the systems were also dependent on the amount of reagents used as well as the sample volume. The dynamic ranges were acceptable for clinical assays for cholesterol. If compared toward the separation capacity of LDL and VLDL, GDS could remove 300 mg/dL in maximum and GIS did 400 mg/dL. The two systems showed fairly good reproducibility of assays with the coefficients of

batch-to-batch variation less than 5%.

References

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