

The Effect of Immunoaffinity Column Chromatography Elution Buffers on HDL Apolipoprotein Detection

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Abstract

High-density lipoproteins (HDL) are complex particles composed of lipids and apolipoproteins. The major function of HDL is to transport cholesterol from peripheral tissues to the liver. HDL apolipoproteins include apoA1, a major structural apolipoprotein; apoCIII, a protein that inhibits binding of apoE and apoB-100 to hepatic receptors; and apoE, a ligand for the low-density lipoprotein (LDL) receptor, apoE receptor, and LDL receptor related protein. Immunoaffinity column chromatography methods are frequently used to isolate HDL subspecies. Therefore, it is important to understand how elution buffers may be affecting protein detection and potentially denaturing proteins. In order to optimize immunoaffinity column chromatography elution conditions, we tested the effects of two elution buffers, acetic acid and sodium thiocyanate (NaSCN), on apoA1, apoCIII, and apoE. We tested the elution buffers for: number of elutions required to elute protein, protein recovery, and reduction of protein detection after treatment with elution buffer. Plasma samples containing known concentrations of apolipoproteins listed were used. This allowed us to compare expected concentrations and measured concentrations after treatment. The sandwich enzyme-linked immunosorbent assay (ELISA) method was used to quantify protein concentrations. The results of these experiments will allow us to maximize protein recovery and account for loss of protein detection due to treatment with elution buffers.