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Application Note

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De-lipidation and de-proteinization or hyperlipidaemic serum samples using Vivaspin®

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Introduction

The major lipids found in blood are cholesterol, triglycerides and phospholipids. Due to their limited solubility in an aqueous environment, lipids are transported in the blood as lipoprotein complexes. Lipoproteins are usually classified according to density differences. These classes are chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL) and high density lipoprotein (HDL). Serum lipids and lipoproteins are important clinically, because hyperlipidemia is a risk factor for disease and hypercholesterolemia is strongly related to coronary heart disease. A very common and effective treatment of lipoprotein disorders depends greatly on diet and lifestyle management. In this study, we evaluated the efficacy of Vivaspin® concentrators for the removal of lipids and proteins from serum samples to permit analysis of low molecular weight species, particularly from patients diagnosed with elevated levels of lipids in serum.

Materials and Methods

Eleven serum samples of approximately 1.5 mL, with triglyceride levels greater than 8.0 mmol/L, were obtained from the Clinical Chemistry department at the Northern General Hospital (Sheffield, UK). A 0.5 mL aliquot of each sample was retained untreated for comparative analysis. Lipids were removed from the remaining 1 mL by filtration through a Vivaspin® 2 device incorporating a 0.2 µm PES microfiltration membrane. A 0.5 mL sample of the filtrate was retained for analysis and the remaining serum was passed through a Vivaspin® 2 concentrator with 10 kDa MWCO PES ultrafiltration membrane, to remove proteins. Removal of lipid was determined by measuring the optical density (at 450 nm) of the samples before and after microfiltration. Removal of protein was determined by measurement of Acid Glycoprotein (AGP), a marker chosen due to its relatively low molecular weight (approximately 45 kDa) amongst serum proteins and the availability of accurate means for its measurement, using a Mira auto-analyser (Roche Diagnostics).

Results

De-lipidation of serum samples by ultrafiltration is a slow but reliable process. A 6 hour total centrifugation time at 2,000 rpm (swing bucket rotor) was required to pass the samples through the 0.2 µm PES membrane. This was expected as the 0.2 µm filter was used to clarify samples rich in visible lipid droplets. Samples were transferred to fresh concentrators after approximately 50 % of the material had been filtered. Approximately 98 % of the clarified material passed through the 10 kDa MWCO ultrafilter following centrifugation at 2,000 rpm for 1 hour. Optical densities were recorded as a measure of lipid level in each sample before and after each filtration step (Table 1). AGP concentrations were recorded as a measure of protein content in each sample before and after each filtration step (Table 2).

Table 1.

Relative lipid content determined by OD measurements of untreated and filtered serum samples at 450 nm.

Sample	Blank	1	2	3	4	5	6	7	8	9	10	11
Untreated	0.192	1.58	1.11	0.41	1.08	1.14	1.06	0.55	1.73	1.58	0.78	0.47
0.2 µm Filtrate	0.186	0.12	0.20	0.13	0.69	0.33	0.09	0.12	0.12	0.21	0.32	0.12
10 kDa MWCO Filtrate	0.050	0.07	0.05	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

Table 2.

AGP concentrations (g/L) in untreated, microfiltered and ultrafiltered serum samples. Measurements were obtained using a Mira auto-analyser.

Sample	Blank	1	2	3	4	5	6	7	8	9
Untreated	0.19	0.93	0.92	0.73	0.86	0.79	1.17	0.99	0.99	0.77
0.2 µm Filtrate	—	0.46	0.47	0.59	0.45	0.37	0.49	0.50	0.44	0.46
10 kDa MWCO Filtrate	—	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Conclusions

The OD data show that the majority of lipids (59-95%, average across all eleven samples = 76%) were removed by filtration through the 0.2 µm membrane filter. In addition, as shown by the AGP data, significant amounts of protein (approximately 50%) were removed during this initial filtration process. It is possible that these proteins were removed with the lipid by adhering to the lipoprotein surface. The OD data also suggest that effectively all protein and lipid material was removed from the samples after the second filtration step when using devices with 10 kDa MWCO PES membranes. The AGP data support this, since there was no detectable AGP after this ultrafiltration step.

We set out to explore whether Vivaspin® centrifugal concentrators could remove lipid and protein from hyperlipidemic serum. The 0.2 µm membrane does remove all particulate material and most lipids. It is recommended that serum containing such particulate material be filtered through a microfiltration membrane with larger pore size (e.g. 0.45 µm) prior to the de-lipidation process described here, to minimize membrane clogging and increase the speed of de-lipidation.

In a second filtration step, a Vivaspin® 2 device with 10 kDa MWCO PES membrane was used to effectively de-proteinate the serum samples, as judged by the removal of proteins with molecular weight of at least 45 kDa (AGP).

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