application note

VIVASCIENCE

De-lipidation & de-proteination of hyperlipidaemic serum - Use of Vivaspin ultrafiltration concentrators to de-lipidate and de-proteinate hyperlipidaemic serum samples

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Background

The major lipids found in blood are cholesterol, triglycerides and phospholipids. Lipids, due to their limited solubility in an aqueous environment 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 hyperlipidaemia is a risk factor for disease and hypercholesterolaemia 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, the efficacy of Vivaspin concentrators for the removal of lipids and proteins from serum samples was evaluated in order to permit analysis of low molecular weight species, particularly from patients diagnosed with elevated levels of lipids in serum.

Materials

Eleven serum samples with triglyceride levels greater than 8.0 mmol/l were obtained from the Clinical Chemistry department at the Northern General Hospital, Sheffield. Vivaspin concentrators (2ml capacity; VS 2, Vivascience, UK) containing 0.2 µm and 10 kDa molecular weight cut-off (MWCO) polyethersulfone membranes were centrifuged in a swing-out rotor. A Mira auto-analyser from Roche Diagnostics was used for measuring serum levels of acid glycoprotein.

Methods

Eleven serum samples of approximately 1500 µl were used for this study; 500 µl of each sample was retained untreated for comparative analysis. The remaining 1ml was passed through a Vivaspin 2 incorporating a 0.2 µm PES microfilltration membrane in order to remove lipids, 500 µl of sample was retained for analysis and the remaining serum was passed through the 10 kDa MWCO ultrafiltration membrane in a Vivaspin 2 concentrator in order to remove proteins. Removal of lipid was determined by measuring the optical density (at 450 nm) of the samples before and after filtration. Removal of protein was determined by means of an assay for Acid Glycoprotein (AGP), a marker glycoprotein chosen due to its relatively low molecular weight (ca. 45kDa) amongst serum proteins and the availability of accurate assays for its measurement.

Results and Discussion

De-lipidation of serum samples by ultrafiltration is a slow, but reliable process. A 6 hour spin at 2,000 rpm was required in order to pass the samples through the 0.2 µm PES membrane. This would be expected as the 0.2 µm filter would be clarifying a sample rich in visible lipid droplets. Samples were transferred to new concentrators after about 50 % of the material had been filtered. Approximately 98 % of the remaining material passed through the 10 kDa MWCO filter 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).

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Table 1. Relative lipid content of untreated and filtered serum samples.

OD 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
a Filtrate 1	0.186	0.12	0.20	0.13	0.69	0.33	0.09	0.12	0.12	0.21	0.32	0.12
b Filtrate 2	0.050	0.07	0.05	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

a After filtration through 0.2 µm membrane

b After filtration through 10 kDa MWCO membrane

AGP concentrations were recorded as a measure of protein content in each sample before and after each filtration step. (Table 2)

Table 2. AGP concentrations of untreated and filtered serum samples.

AGP (g/l)

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
a Filtrate 1	---	0.46	0.47	0.59	0.45	0.37	0.49	0.50	0.44	0.46
b Filtrate 2	---	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

a After filtration through 0.2 µm membrane

b After filtration through 10 kDa MWCO membrane

The OD data show that a large majority of the lipid does appear to be removed by filtration through the 0.2 µm membrane filter. However, 10-20 % of the lipid could not be recovered. In addition, as shown by the AGP data, significant amounts of protein are lost by this process. It is possible that the proteins simply adhere to the lipoprotein surface and are removed with the lipid. The OD data also suggest that all protein and lipid material is removed from the sample after filtration using a 10 kDa MWCO PES membrane. The AGP dat a support this since there is no detectable AGP after the second filtration step.

Conclusion

The remit of this work was to explore whether the Vivaspin centrifugal concentrators could remove lipid and protein from hyperlipidaemic serum. The 0.2 µm membrane does remove all particulate material and lipids.
It is recommended that serum containing such particulate material be filtered through a larger MWCO membrane filter (ca. 0.45 µm filter) prior to the de-lipidation process in order to minimize membrane clogging and increase the speed of the de-lipidation experiments.
The 10 kDa MWCO ultrafiltration concentrator appears to successfully de-proteinate serum samples.
The device has been shown to remove proteins of at least 45 kDa (AGP).

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