Procedure

We decided initially to immunodeplete the samples on a MARS 14 column using an akta FPLC system. The latter was only installed five days before the study deadline and the fraction collector malfunctioned during the run, resulting in the loss of 50% of the samples.

50% of the remainder (undepleted plasma) was in solution digested with Endo Lys C, followed by trypsin and one half of the resulting peptide mixture was analysed on a Bruker HCT Ultra using an “MRM” programme, acquiring MSMS data for 8 different peptide ions, 4 for each of the proteins. Parent ions were selected using a combination of the information supplied with the samples by ABRF and data from gpmdb. The other half was analysed on a Applied Biosystems Qtrap 4000 in MRM mode, selecting the same 8 peptides and aquiring 2 transitions for each peptide.

1 h was spent on the analysis of the raw data and we could not find evidence for the presence of any of the chosen peptides in data files from either of the two mass spectrometers. This is most likely due to suppression from abundant plasma proteins – we assume that depletion would have been the way to go.