REDUCING HIGH ABUNDANCE PROTEINS IN HUMAN SERUM: DEPLETION VERSUS EQUALIZATION

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Since protein depletion is becoming a common choice as the first step in biomarker discovery this work highlights the importance of understanding that different regions of serum are interrogated as a function of the strategy chosen to perform the depletion. This fact becomes critical in studies about sample profiling using peptide fingerprinting where de presence of high abundant proteins will dominate the spectrum and will suppress the signal of the less abundant ones, thus making more difficult the successful use of statistical tools to clusterize samples.

In this work two methods to avoid major sera proteins in human serum have been studied and compared. Protein depletion with acetonitrile and protein equalization with the ProteoMiner (Bio-Rad) have been assessed by 1D-gel electrophoresis and mass spectrometry, given special attention to reproducibility, handling and profile of depletion. After treatment 5 and 9 major proteins (top 20) and 16 and 20 proteins belonging to the low abundant fraction were indentified by acetonitrile, and ProteoMiner methods respectively. We have found that each method provides a different pattern of depletion: the ProteoMiner method provided a compression of the dynamic range of serum protein concentrations whilst acetonitrile method allows an effective depletion of the protein fraction above 72kDa.

Therefore, if there is no guidance in biomarker research, the best choice seems to use different strategies in the same study in order to guarantee that the serum proteome is interrogated in the larger extent possible.