METHODS – ITRAQ PROTEIN ANALYSIS

ITRAQ labeling (isobaric tagging for relative and absolute quantitation, Applied Biosystems) is an approach to analysis of the relative abundance of proteins in different samples. In this method, samples of interest are reduced and alkylated per standard protocols. Following this, each sample is digested with trypsin and the tryptic peptides are labeled with a unique ITRAQ tag. These are low molar mass charged isobaric tags (MW 114, 115, 116, and 117 daltons) that have peptide reactive groups which bind the peptide N-terminus and lysine side chains. Following ITRAQ labeling, the study samples are combined, peptide sequencing is performed using mass spectrometry, and proteins identified. In addition to identification of proteins present in the test samples, the unique ITRAQ label quantifies the relative difference of the abundance of proteins between samples. The protein abundance is generally expressed as a ratio of relative abundance between the samples compared. The ITRAQ approach is able to reliably detect differences in protein abundance of greater than 50%. ITRAQ labeling is therefore used for measurement of relative protein abundance and there are a number of reports of its use in the literature.

RESULTS – ITRAQ PROTEIN ANALYSIS

Table 1. Identification of the thirty spots with the greatest volume differences from the depleted 2D DIGE gels shown in Figure 5. Many of the spots identified were multiple peptides from one originating protein, with serum albumin and apolipoprotein A-I being the greatest sources.

RESULTS – 2D DIGE GEL ANALYSIS

Figure 4: Average log (2) diff-score for 26 most abundant proteins in plasma (S/D plasma versus Resusix)

Dehydration of plasma greatly increases the ease of its transport and length of storage, and has been practiced in several forms since World War II. However, lyophilization of plasma may induce changes in proteins or other constituents, as evidenced in turbidity at activities after infusion in a swine resuscitation model. Here is presented initial proteomic year clinical and economic success story. Transfus Apher Sci 2003; 28:93–100.

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(6) The initial gel was dominated by the plasma

(7) The initial gel was dominated by the plasma