A PROTEORED MULTI-CENTRIC STUDY TO ASSESS REPRODUCIBILITY OF RELATIVE QUANTIFICATION BY LC-MS PROTEOMIC ANALYSIS

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Diverse LC-MS based strategies are being used for quantitative differential analysis of proteomes, including a variety of isotopic labeling reagents, and label-free approaches. In order to evaluate the performance of different quantitative methods, and their reproducibility across laboratories, we set up a multi-centric study, carried out at 22 laboratories, most of them members of the ProteoRed network.

Participant labs received two test samples consisting on an identical matrix of cytoplasmatic E. Coli proteins, of medium complexity (ca. 300 proteins), to which four standard mammalian proteins had been spiked in different amounts, ranging from the fmol to the pmol level per µg of total protein. The ratios of the spiked proteins between the two samples were chosen to range from 1.5:1 to 5:1. The reported results of the differential analysis corresponded to analysis using the isobaric reagents iTRAQ (11 data sets) or TMT (4 datasets), the non-isobaric ICPL (2 data sets), and different label-free approaches (5 datasets). Additionally, 3 laboratories performed also 2D-gel DIGE analysis. In all cases four technical replicas of each sample were analyzed.

The results demonstrate a good within lab and across lab general reproducibility, irrespective of the method of analysis and the spectrometer used, with the exception of the least abundant spiked protein (at levels of 1-5 fmol per µg of total protein). Within lab, the average %CV of the measured ratios is around 9% (protein spiked at 1pmol/µg), 12% (200fmol/µg), 12% (25fmol/µg) and 7% (1fmol/µg). Across labs %CVs of the measured ratios are 12.5%, 22%, 22%, and 50% for the four spiked proteins, respectively. Accuracy of the measured ratios compared to the theoretical values averages 89%, 80%, 80% and 70%, respectively.

The results of this multi-centric study demonstrate the reproducibility and consistency of relative quantification measurements, by different analytical approaches and at different laboratories.