REFERENCE MATERIALS FROM HUMAN BODY FLUIDS: QUANTITATIVE WORKFLOWS FOR BIOMARKER DISCOVERY AND VALIDATION USING TANDEM MASS TAGS

SCHULZ-KNAPPE P., PIKE I., KUHN K.

Proteome Sciences plc, Cobham, UK.

Proteomics is considered as key technology for peptide and protein biomarker discovery and development. In the last years, limited reproducibility of the methods and for qualitative and quantitative proteome analysis has restricted discovery and especially validation of biomarkers. Comparison of results between different studies and labs is often not possible.

Clinical laboratories typically use reference materials to calibrate and certify assays. By using reference materials instruments are qualified and entire workflows are benchmarked. Unfortunately, proteomics lacks such materials to compare results within labs and between different groups.

One of the new strategies for quantitative proteomics is to use mass spectrometry combined with isobaric label reagents. We have introduced different sets of tandem mass tag (TMT®) reagents for such purposes. All tags label amino groups at N-termini and lysine residues. The tags have the same overall mass and identical samples, the samples can be mixed and processes simultaneously. Labeled peptides and proteins behave identically during all steps as sample handling and chromatography so that the quantitative ratio between individual proteins remains constant after mixing of samples. This conservation of relative proportions is a key for subsequent separation and analysis. Since the mass tags release individual reporter ions during tandem mass spectrometry, peptides and proteins can be quantified specifically.

Here we present the utility of TMT® for the development of biological reference materials for body fluids such as blood plasma, urine, and others. The entire protein composition of a body fluid is labeled in bulk quantities, either by intact protein labeling or after tryptic digestion, to serve as a defined proteome reference. By using different tags from our TMTsixplex™ set, calibration mixes such as 1:2:4:8 ratios can be produced. An aliquot of the reference sample is spiked into all samples of a discovery or validation study. Using this as reference enables scientists to have quality control and share their results worldwide, regardless of instrumentation and workflow design.