

# Biological reference materials for proteomics: Tandem Mass Tag-labeled reference materials and their utility for mass spectrometry based multipoint calibration and quantification

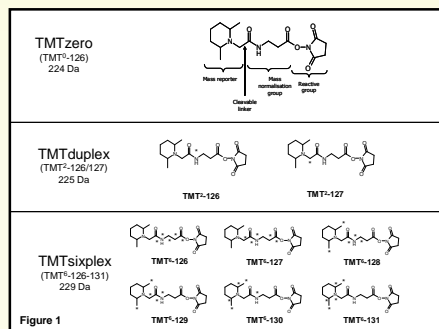
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## Overview

- Reference materials are routinely used in many applications in clinical chemistry to ascertain adequate metrological performance of analytical tests between labs and over long time periods.
- The utility of proteomic analyses has been limited by compromised precision and poor reproducibility of qualitative and quantitative techniques.
- We develop novel biological reference materials for proteomics analysis by tagging reference materials with Tandem Mass Tags (isobaric mass labels, TMT). These references are spiked into individual study samples to provide calibration curves and relative quantification of the entire sample proteome with highly improved precision and coefficient of variation.

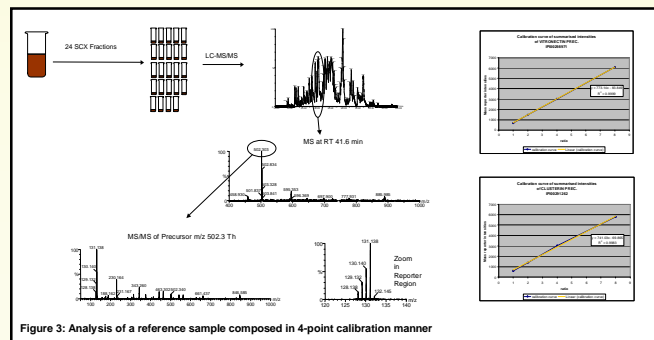
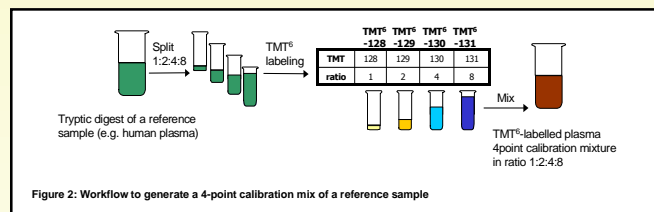
## Tandem Mass Tags

- TMTs are an innovative set of isobaric mass tags for labeling proteins and peptides at amine functions and mixing of up to six different protein samples (Fig. 1). During MS/MS, tags give rise to six different reporter ions from 126 to 131 Da thus allowing (relative) quantification. In TMTsixplex, each tag adds a mass of 229 Da per labeled amine to the protein. TMTduplex (TMT<sup>2</sup>) and TMTzero (TMT<sup>0</sup>) share the TMT<sup>5</sup> structure. They add 225 and 224 Da per label, whilst reporter mass is 126/127 (TMT<sup>2</sup>) and 126 (TMT<sup>0</sup>).



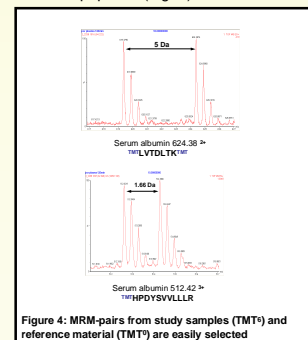
## TMT-labeled reference materials

- Biological materials (Plasma and cerebrospinal fluid) were labeled with different TMTs, with and without depletion of high abundant proteins, usually after trypsin digestion in order to serve different reference material workflows.
- In one representative set of experiments, a plasma protein tryptic digest stock solution (Fig. 2) was split into four aliquots which were labeled with TMT<sup>5</sup> reagents 128, 129, 130 and 131 and combined in a 1:2:4:8 ratio to form a 4-point calibration reference material. Strong cation exchange separation was followed by reversed phase-MALDI ToF/ToF analysis (ABI 4800) as well as LC-ESI-QTOF analysis (Waters QTOF II, Fig. 3).
- The analysis with the Q-ToF workflow gave 3965 MS/MS spectra of which 1015 gave identification of peptide sequences. Calibration curves of reporters 128/129/130/131 in the identified spectra gave very good quantification criteria with R<sup>2</sup> values above 0.95 in 80% of cases, covering several logs of concentration. Using MALDI ToF/ToF, 15,528 MS/MS spectra were accumulated of which more than 50% had a R<sup>2</sup> value better than 0.99. 12,000 spectra demonstrated quantitation properties with deviations less than 10% from the expected value.
- Variations of the setup such as single-point calibration, alterations in the calibration curve spread and use of TMTzero-labeled reference materials demonstrate equivalent results.



## Assay development

- Peptides labeled with different TMTs show identical chromatographic retention times as well as identical ionisation and fragmentation behaviour of tags and tagged species.
- This allows for a seamless move from discovery through validation and routine assay development. In addition to the use of multipoint-calibration during MS/MS the move to MRM-based quantitation is possible using TMTzero-labeled reference material (Delta Mr: 5 Da per Tag). It serves as the universal source for isotopically labeled quantification partners for TMTsixplex-labeled study samples.
- Prior MS/MS experiments are interrogated to select proteotypic peptides for proteins of interest which show favourable elution and fragmentation behaviour and deliver useful fragment ions for MRM. Quantitation of the TMTsixplex peptide against the reference material labeled with TMTzero is easily possible. This allows to quickly use the selectivity and specificity of MRM combined with TMT-labeling. The biological reference material serves as universal source of calibration and quantification partners for MRM. Setup of multiplex assays is possible without the need to synthesize isotopically labeled reference peptides (Fig. 5).



## Conclusions

- Tandem Mass Tag-labeled reference materials support discovery and validation of protein biomarkers.
- These reference materials improve quantification, reproducibility and comparability of proteomics studies.
- Relative quantification of hundreds of proteins in samples of interest is achieved by referencing against specific reporter ion calibration curves from reference material labeled with TMT reagents.
- Transition into highly specific MRM-type validation assays is fast and easy by utilising the isotopic TMTzero as reference partner.

## References

[1] Thompson A, Schäfer J, Kuhn K, Kienle S, Schwarz J, Schmidt G, Neumann T, Harmon C.: Tandem Mass Tags: A Novel Quantification Strategy for Comparative Analysis of Complex Protein Mixtures by MS/MS. Anal. Chem. 2003, 75, 1895-1904.  
 [2] Dayon L, Hainard A, Licker V, Turck N, Kuhn K, Hochstrasser DF, Burkhard PR, Sanchez JC.: Relative Quantification of Proteins in Human Cerebrospinal Fluids by MS/MS Using 6-Plex Isobaric Tags. Anal. Chem. 2008, 80, 2921-2931

