URINE SAMPLE PREPARATION PROTOCOL STANDARDIZATION FOR SELECTED REACTION MONITORING (SRM) BASED ASSAY

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Urine has become one of the most attractive bio-fluids in clinical proteomics, as it can be obtained non-invasively in large quantities and is stable compared to other bio-fluids. The urinary proteome has been studied by almost all proteomics technologies, but MS-based urinary protein and peptide profiling has emerged as most suitable for clinical applications.

Selected Reaction Monitoring (SRM) based assay is a technology that ideally complements the discovery capabilities of shotgun strategies through its unique potential for the reliable quantification of low abundance analytes in complex mixtures, such urine samples. While SRM shows considerable promise for protein quantitation, the technique is still in its infancy for clinical applications. Biomarker discovery approaches in urine have been hindered by concerns for reproducibility and inadequate standardization of proteomics protocols. Therefore, a robust analytical protocol for sample preparation of urine sample is needed to obtain meaningful proteomic analyses.

The aim of this study is to standardize the urine sample protein extraction protocol in order to enable quantitative protein measurements in urine samples. We evaluated the sample preparation protocol using the quantification of spiked exogenous proteins as internal standard. Addition of yeast proteins at the beginning of sample preparation and corresponding labeled yeast peptides before the LC-MS experiments in SRM mode have been used to account for all sources of sample loss during the entire analytical process by studying the reproducibility and recovery based on the quantification by SRM.