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Inter-laboratory evaluation of instrument platforms and experimental workflows for quantitative accuracy and reproducibility assessment

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ABSTRACT

The reproducibility of plasma protein quantitation between laboratories and between instrument types was examined in a large-scale international study involving 16 laboratories and 19 LC–MS/MS platforms, using two kits designed to evaluate instrument performance and one kit designed to evaluate the entire bottom-up workflow. There was little effect of instrument type on the quality of the results, demonstrating the robustness of LC/MRM-MS with isotopically labeled standards. Technician skill was a factor, as errors in sample preparation and sub-optimal LC–MS performance were evident. This highlights

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the importance of proper training and routine quality control before quantitation is done on patient samples.

1. Introduction

Reproducible and accurate quantitation is a requirement for clinical and translational applications in targeted proteomics. The "gold standard" method in MS-based proteomics relies on multiple reaction monitoring (MRM) with stable isotope-labeled standards (SIS) incorporated within a bottom-up workflow [1]. Although MRM with labeled standards has been used for decades in small-molecule analysis (*e.g.*, hormones, drugs) [2,3], the use of this technique in proteomics introduces additional analytical variables related to sample preparation (*e.g.*, digestion) that increases the complexity of the sample analysis, thereby increasing the potential for interferences with the MRM transitions. To address this, standardization of proteomics methods has been encouraged by the Human Proteomics Proteome Organization's Plasma Proteome Project [4–10] and supported further by the US National Cancer Institute through the Clinical Proteomic Tumor Analysis Consortium [11,12]. This is necessary to enhance the global reproducibility of high quality data using different MS technologies.

As part of this standardization effort, we previously developed three standardization kits for instrument QC on a daily (Kit A) or monthly (Kits B and C) basis for LC–MS/MS platform (Kits A and B) and complete workflow (Kit C) assessment [13,14]. These kits contain a set of materials and analysis tools that enable value tracking and accuracy estimation by comparison with a set of reference values/ranges. Lyophilized pre-digested plasma standards that were spiked with a SIS peptide mixture (43 peptides from 43 human plasma proteins) are provided in Kits A and B, while raw starting materials (namely undepleted plasma, trypsin, and the SIS mix) are provided in Kit C for sample preparation and subsequent processing/analysis. In all cases, samples are processed by LC–MS/MS in a targeted or semi-targeted manner (*i.e.*, by using MRM on a triple quadrupole mass spectrometer or by using parallel reaction monitoring (PRM) on a hybrid quadrupole-Orbitrap mass spectrometer, respectively). These operative modes differ in that precursor-product ion pairs (*i.e.*, transitions) are sequentially monitored in MRM, whereas in PRM, full product ion spectra are collected from the collisional fragmentation of all target precursors in a given *m/z* window (see Fig. 1a and b in [15] for comparative schematics).

In this paper, we report the use of these three kits to evaluate the accuracy and reproducibility of a quantitative proteomics analysis of 43 high-to-moderate abundance plasma proteins in a bottom-up workflow, and to determine the source of errors if sub-

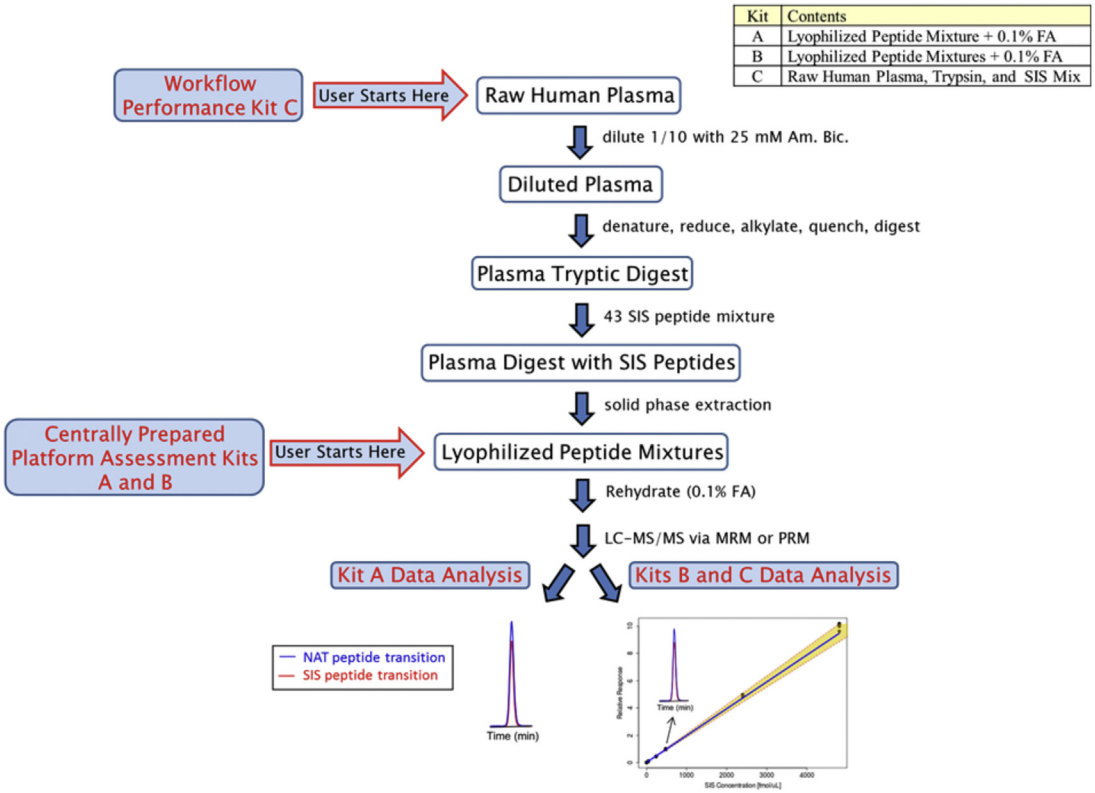

Fig. 1. Experimental workflows and starting points for the 3 QC kits. Kits A and B required simple rehydration of the lyophilized peptide mixture(s) prior to sample processing by LC/MRM-MS or LC/PRM-MS, whereas Kit C required the user to execute the entire workflow from 3 supplied starting materials (namely plasma, trypsin, and the SIS mix). Kit dispersal and data/quantitative analysis were performed at the UVic-Genome BC Proteomics Centre. Quantitation was facilitated by Qualis-SIS which generated standard curves (relative response vs. SIS concentration) from the SIS (red trace) and NAT (blue trace) response data of each peptide's quantifier transition. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)