Proteomic Analysis – Sample Preparation and Multiplexing for Relative Quantitation

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ABSTRACT
Purpose: Determination of abundant proteins is required to identify and measure changes in patients diagnosed with pulmonary hypertension (PH). Therefore, we aimed to establish a multiplexed approach for relative quantitation (RQ) of high abundance plasma proteins using TMT labeling.

Methods: Plasma samples were subjected to titanium dioxide (TiO2) depletion and fractionated into normal and PH-HFpEF (high blood pressure associated with diastolic dysfunction) subgroups. Blood plasma samples (n = 30) were labeled with TMT reporter ions 11-plex™ and analyzed by Thermo Scientific™ Proteome Discoverer 2.3 software. Protein abundance was quantified using the ratio of signal intensity of the reporter ion peaks from the normal_1 subgroup with respect to the internal standard (IS).

RESULTS
In brief, we have established a workflow for multiplexed quantitation of high abundance plasma proteins for PH-HFpEF patients. This workflow, in addition to being reproducible, allows for the accurate and efficient quantitation of proteins that are highly abundant, which are often difficult to detect in biological samples. The workflow involves the use of TiO2 depletion to remove low-abundance proteins, followed by TMT labeling and quantitation using Proteome Discoverer software. This approach offers several advantages, including reduced sample consumption, increased throughput, and improved accuracy and reproducibility for high abundance proteins. The workflow can be used in various clinical and research settings to study disease progression and identify potential biomarkers for PH-HFpEF.

CONCLUSIONS
100 Reproducible depletion of abundant proteins is obtained by using Thermo TiO2 TiO2, abundant protein depletion column. Reproducible and quantification of high abundance proteins is required to identify and measure changes in PH-HFpEF patients. We have established a multiplexed approach for RQ of high abundance plasma proteins using TMT labeling.

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