

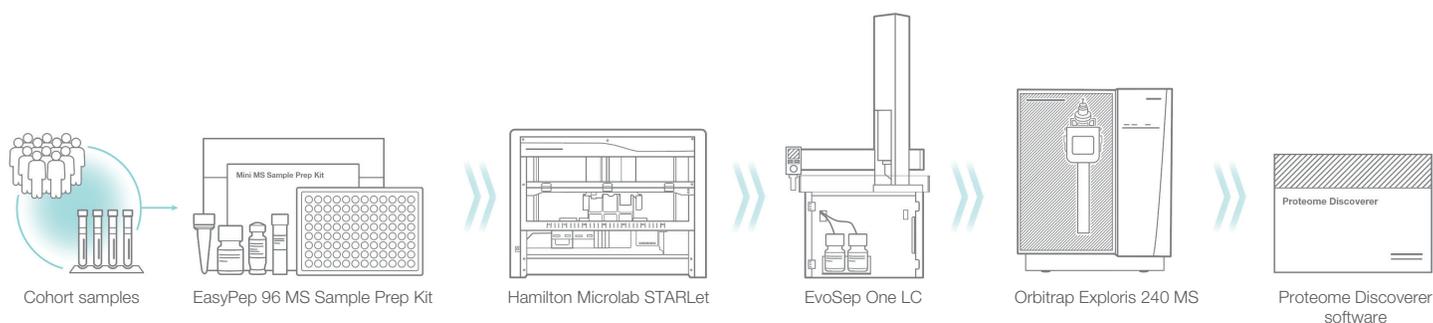
## Orbitrap Exploris 240 mass spectrometer delivers significantly increased lab productivity and reproducibility of human plasma proteome profiling from large human cohort studies

“For large clinical cohort studies where hundreds of plasma samples are to be analyzed, a robust, reproducible and automated sample preparation and analysis workflow is highly desirable. The uHTPPP workflow<sup>1</sup> meets our needs, which include a 96-well plate formatted protein digestion kit with all protein digestion reagents included, a script automating the entire digestion process from plasma sample dilution to concentrating peptides, and a plasma proteome profiling method template for MS analysis.”

— Dr. Qibin Zhang



Blood (plasma or serum) is the most accessible specimen for clinical proteomics research. Key challenges associated with proteomics analysis of plasma proteins are the reproducibility and throughput of the workflow. The protocol is long and labor intensive and oftentimes the technical variability may exceed the biological variability, which reduces the confidence of the biological findings when small changes at the protein level are expected for chronic diseases.



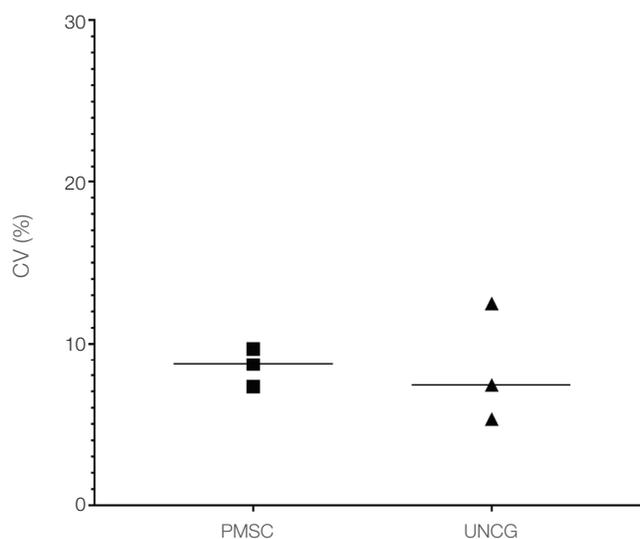
**Ultra-high throughput plasma protein profiling (uHTPPP) workflow using the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer.**

Thermo Fisher Scientific developed a standardized and scalable mass spectrometry-based workflow for human blood focusing on balancing the depth of identification and the scalability for sampling large population cohorts. The uHTPPP workflow consists of an automated sample preparation method and analysis of samples on an EvoSep One LC system coupled to an Orbitrap Exploris 240 mass spectrometer using the Thermo Scientific™ EASY-Spray™ ionization source and a Thermo Scientific™ EASY-Spray™ HPLC column. An automated sample preparation solution was designed to integrate the Thermo Scientific™ EasyPep™ 96-well MS Sample Prep Kit so the entire workflow can be easily implemented to conduct large-scale, human cohort plasma protein profiling studies.

The priority of the University of North Carolina at Greensboro (UNCG) team was to develop an analytical method to analyze hundreds of plasma samples using HRAM-MS. They have utilized Thermo Scientific™ Orbitrap™ mass spectrometers for many years for biomedical research, but the demand to accelerate biomarker discovery efforts for unmet medical needs leads to an increase in sample size from human cohorts and requires methods that can deliver reproducible qualitative and quantitative results within a short period of time. There is also the desire to streamline and standardize the workflow to perform routine analysis of human plasma proteins, Dr. Zhang explained, “I think key challenges associated with proteomics analysis of plasma proteins are the reproducibility and throughput of the workflow. We need a reproducible sample preparation solution, robust LC-MS platform, and standardized data acquisition as well as data analysis methods to produce precise quantitative comparisons among hundreds and potentially thousands of human samples. This is a considerable amount of work.”

**Reproducibility of the automated sample preparation solution**

It is critical to demonstrate good inter-laboratory and intra-laboratory reproducibility of the analytical workflow for large-scale studies. Here we compared results from processing lab generated QC samples using the EasyPep 96-well MS sample prep kit and the automation script provided by the Precision Medicine Science Center (PMSC) and UNCG labs. Peptide concentrations from QC serum or plasma samples were used to assess the reproducibility of an automated sample preparation solution performed in these two laboratories. As shown in Figure 1, on average 8.6% CV was observed from processing QC serum samples from three independent experiments by the PMSC, and 8.4% CV was observed from processing QC plasma samples from three independent experiments by the UNCG lab. These results showed excellent repeatability within each laboratory and good reproducibility between these two laboratories.



**Figure 1. Box plots summarize % CV from three independent experiments from the PMSC and UNCG using lab-generated QC serum or plasma using the uHTPPP workflow automated sample preparation solution.**

“The uHTPPP workflow was easy to implement. The EasyPep 96-well MS kit has very detailed instructions, the Hamilton automation script is easy to operate and Orbitrap Exploris 240 MS is a robust MS instrument. Previously it would take us more than one month to do plasma profiling of 300 samples, now we can do it routinely within two weeks.”

— Dr. Qibin Zhang

### Reproducibility of results from the standard plasma profiling MS method from the Orbitrap Exploris 240 mass spectrometer

In addition to preparing high quality samples in a reproducible manner, we also compared the reproducibility of the LC-MS performance between these two laboratories. Using the commercial HeLa digest standard, 60 samples per day (SPD) throughput of the Evosep One LC, the “plasma profiling” template method from the method editor of the Orbitrap Exploris 240 MS, and a matching database search method from Thermo Scientific™ Proteome Discoverer™ software, we compared protein and peptide identifications generated from the systems in the PMSC and in the UNCG labs. As shown in figure 2, less than 5% CV was observed from protein and peptide identifications between experiments performed in the PMSC and the UNCG labs. Again, these results showed excellent repeatability within each laboratory and good reproducibility between these two laboratories. “Our experience with the OE240 has been very positive. We

have run >1000 plasma samples since we acquired the instrument last July, most of them were run using the Evosep LC at 300 ng loading. We have not done any deep cleaning because we did not see any noticeable signal deterioration. The only thing we did through this time was cleaning the inlet transfer tube and calibration” said Dr. Qibin Zhang.

### Conclusion

Variability and dynamic range of protein abundance significantly impacts analysis of the human plasma proteome. To develop novel markers indicative of diseases from human blood using mass spectrometry-based approaches, the workflow must be robust and reproducible for hundreds or thousands of samples to make a reliable conclusion from translational studies. The uHTPPP workflow utilizing the Orbitrap Exploris 240 mass spectrometer presents a standardized, high-throughput, and reproducible workflow for blood-based biomarker discovery. This workflow brings significant advantages over other platforms including:

- Easy to follow automation script with minimal hands on time and low variability among samples.
- Standardized methods with commercial reagent kits, data acquisition method template, and data search strategy.
- Significant increase in lab productivity and throughput of sample preparation for mass spectrometry-based plasma proteome analysis.
- Low analytical variability with robust LC and MS systems.
- Excellent inter-laboratory and intra-laboratory reproducibility for large-scale studies.

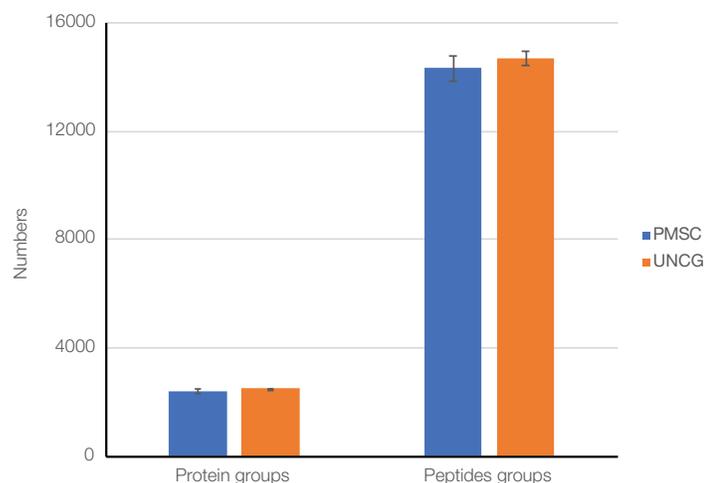


Figure 2. A comparison of protein and peptide identifications using the uHTPPP workflow from the PMSC and UNCG labs.



### About Qibin Zhang

Dr. Qibin Zhang is an Associate Professor in the Department of Chemistry and Biochemistry, and Co-Director of the Center for Translational Biomedical Research at the University of North Carolina at Greensboro, U.S.A. He obtained his PhD degree in Chemistry from the University of California at Riverside, followed by postdoctoral studies in proteomics and metabolomics at the Pacific Northwest National Laboratory (PNNL), U.S. Department of Energy. He started his independent research career at PNNL as a Scientist and moved to the current position in 2014. With multiple grants support from the National Institutes of Health and the American Heart Association, the Zhang lab is focused on development of advanced mass spectrometric analysis tools and clinical applications of proteomics, lipidomics and metabolomics to diabetes, diabetic complications and liver diseases.

### The Center for Translational Biomedical Research at the University of North Carolina at Greensboro

The Center for Translational Biomedical Research (CTBR) at the University of North Carolina at Greensboro is located at the North Carolina Research Campus in Kannapolis, NC. With multiple grants support from the National Institutes of Health and the American Heart Association, one of the primary research focuses of the Center is on disease biomarkers, in particular discovery of protein biomarkers of Type 1 diabetes and diabetic complications for early-stage disease diagnosis and understanding the pathogenic mechanisms for better management and cure of these disorders.

All of these benefits enable translational and clinical research laboratories to process and analyze hundreds and thousands of human blood samples in a fraction of the time compared to the conventional workflow.

### References

1. Trusiak, S.; Snovidia, S.; Patel, B.; Bomgarden, R.; Chen, E., Sampling the Human Population – Ultra-High Throughput Plasma Protein Profiling (uHTPPP) Sample Preparation for Translational Proteomics, Thermo Fisher Scientific Application Note # 65727, 2020.

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