Syllabus

Clinical Proteomics 202: MS-based Precision Diagnostics by Molecular Protein Analysis

Block	Subjects	Learning objectives
1: Rationale for the quantification of proteoforms	Development of clinical chemistry tests based on Test evaluation framework	- Know when and how to develop new clinical chemistry tests - Understand precision diagnostics and personalized medicine - Know how to implement new test into clinical care pathway
	What are proteoforms	- What alterations may affect proteins (PTMs, mutations, splicing)
	Why measure proteoforms	- Understand that proteoforms can affect 'traditional' test results - Understand that proteoforms can affect clinical phenotype
	Interactive session 1	
2: Strategies for the identification and quantification of proteoforms in clinical samples	How to quantify proteins in biofluids	- Know the basics of protein quantitation using LC-MRM-MS o Bottom-up proteomics o Transition development o Protein digestion o Internal standardization
	How to identify and quantify proteoforms – Untargeted	- Know strategies for untargeted analysis of proteoforms - Understand the pros and cons of an untargeted approach - Know how to apply this in practice
	How to identify and quantify proteoforms – Targeted	- Know strategies for targeted analysis of proteoforms - Understand the pros and cons of a targeted approach - Know how to apply this in practice
	Application 1: Apolipoprotein E phenotyping	
3: Advanced topics	How to identify proteoforms – PTMs	- Know the various PTMs that may cause proteoform variation - Know how to adapt an MRM-method to quantify these PTMs
	Quality Controls , Calibration & standardization	- Know how to select quality control materials for clinical proteoform tests - Understand considerations for selection of calibrators for proteoform tests - Know the concept of standardization of clinical chemistry tests, and the potential impact of proteoforms on standardization
	Application 2: Antithrombin proteoforms	

Summary:
Did you know proteins may exist in hundreds of molecular proteoforms? And that each specific proteoform may have different functionality, potentially leading to a pathophysiological clinical phenotype ? How could we measure such proteoforms using mass spectrometry? And how could measurement of proteoforms aid in precision diagnostics?

In this course, we will explain what proteoforms are, and why they may be relevant to measure in a medical laboratory. We will use *real-lab* examples of proteoforms known to affect the patients' health status and guide you through the potential methods on identifying and characterizing proteoforms with multiple-reaction-monitoring MS. We will start the course with the rationale on when and how to develop new diagnostic tests. We will explain the diversity in proteoforms, with a focus on proteoforms caused by mutations, but we will also touch upon PTM-induced proteoforms. Lastly, we will discuss several quality related aspects of these tests. In the end, our aim is to provide the knowledge necessary to apply proteoform analysis by MS in your own (clinical) laboratory.

The course will consist of theoretical background, examples of applications and interactive sessions. A background in quantitative proteomics is helpful but not required. You will need to know the principles of LC and QQQ analysis through multiple reaction monitoring. At the end of the course, you will know why molecular protein analysis could be beneficial and how you can apply it in your laboratory.

Learning objectives:

1. Discuss what proteoforms are and why they may be relevant to quantify.
2. Discuss how the analysis of proteoforms will contribute to precision diagnostics and how clinical care pathways may be altered based on molecular protein measurements.
3. Discriminate how to discriminate proteoforms using multiple-reaction-monitoring mass spectrometry.
4. Evaluate how to evaluate molecular MS data and provide answers for laboratory specialists
5. Ensure to ensure performance and quality of proteoform-based tests.