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## **Preface: It's Mass Spectrometry's Turn to Change Clinical Practice** ix

Geza S. Bodor

## **25-Hydroxyvitamin D Testing: Immunoassays Versus Tandem Mass Spectrometry** 439

Uttam Garg

Vitamin D has been associated with many health conditions. Because of widespread deficiency in the general population, laboratory testing of vitamin D has increased exponentially in recent years. Currently, 25-hydroxyvitamin D (25[OH]D) is considered the best marker of vitamin D status. Automated immunoassays and tandem mass spectrometry are the most widely used assays for the measurement of 25(OH)D. Because a medical decision of vitamin D deficiency and treatment are made based on specific levels, it is important that different 25(OH)D assays are harmonized. Despite standardization efforts, significant differences remain among various methods and laboratories for the measurement of 25(OH)D.

## **Pain Management Testing by Liquid Chromatography Tandem Mass Spectrometry** 455

Geza S. Bodor

For appropriate pain medication monitoring, the analytical method must be sensitive enough to detect the prescribed medication and metabolites at a sufficiently low concentration to recognize compliance, even with a low-dose prescription. The method must also provide excellent selectivity to identify simultaneously present drugs even with similar chemical structures. The analytical method should uncover common illicit drugs/nonprescribed medications. Traditional immunoassays cannot satisfy these criteria, but liquid chromatography tandem mass spectrometry can. It requires expensive instrumentation, careful test design, and extensive validation and produces a large amount of data that must be interpreted according to the clinical context.

## **Matrix-Assisted Laser Desorption Time of Flight Mass Spectrometry** 471

Donna M. Wolk and Andrew E. Clark

Matrix-assisted laser desorption time of flight mass spectrometry (MALDI-TOF MS), adapted for use in clinical microbiology laboratories, challenges current standards of microbial detection and identification. This article summarizes the capabilities of MALDI-TOF MS in diagnostic clinical microbiology laboratories and describes the underpinnings of the technology, highlighting topics such as sample preparation, spectral analysis, and accuracy. The use of MALDI-TOF MS in the clinical microbiology laboratory is growing, and, when properly deployed, can accelerate diagnosis and improve patient care.

**Proteoform Analysis to Fulfill Unmet Clinical Needs and Reach Global Standardization of Protein Measurands in Clinical Chemistry Proteomics** 487

Yuri E.M. van der Burgt and Christa M. Cobbaert

In clinical testing of protein markers, structure variants of the measurand are often not taken into account. This heterogeneous character of protein measurands in immunoassays often renders test standardization impossible. Consequently, test results from different methods can lead to underdiagnosis or overdiagnosis and, thus, undertreatment or overtreatment of patients. The systematic structural analysis of protein isoforms has been coined proteoform profiling and is performed through mass spectrometry-based proteomics strategies. Knowledge on proteoforms allows refining existing unimarker tests and moreover has great potential to contribute to the urgent need for new tests to predict prognosis and severity of diseases.

**Harmonization of Liquid Chromatography–Tandem Mass Spectrometry Protein Assays** 499

Alan L. Rockwood, Mark S. Lowenthal, and Cory Bystrom

Harmonization of diagnostic test results is fundamental to the effective use of laboratory testing in the diagnosis, treatment, and monitoring of disease. Formal approaches to harmonization and standardization provide a rigorous and high-quality roadmap to this end, although the formal harmonization process can be long and complex. In the meantime, more informal approaches to harmonization can provide a useful pathway to improved harmonization in the short term. Factors relevant to harmonization are discussed with particular attention to protein assays using LC-MS/MS. Published formal and informal harmonization projects are provided as examples, including lessons drawn from these projects.

**Accreditation and Quality Assurance for Clinical Liquid Chromatography–Mass Spectrometry Laboratories** 515

Kara L. Lynch

For mass spectrometry (MS) testing in the clinical laboratory, postimplementation monitoring for quality is just as important as method development and validation but often receives less attention. Quality-assurance monitoring for liquid chromatography–tandem MS (LC-MS/MS) testing should be proactive rather than reactive and should monitor the entire testing process. An LC-MS/MS quality-assurance plan should cover overall batch review parameters, individual peak review parameters, system and reagent changes, and assessment of long-term accuracy. This article discusses Clinical Laboratory Improvement Amendments' regulations as they apply to LC-MS/MS-based testing and reviews available guidelines for LC-MS/MS quality assurance and postimplementation monitoring.

**Liquid Chromatography–Mass Spectrometry Education for Clinical Laboratory Scientists** 527

Judith A. Stone and Robert L. Fitzgerald

This article describes the need for, stratifies the complexity of, and proposes detailed lists of training competencies for diagnostic laboratory

personnel using quantitative liquid chromatography–tandem mass spectrometry (LC-MS/MS) for patient care. Although quantitative LC-MS/MS is evolving toward greater automation with less need for technical expertise, gaps remain in resources for training and assessment.

**Special Considerations for Liquid Chromatography–Tandem Mass Spectrometry Method Development** 539

Brian A. Rappold

Method development for diagnostic liquid chromatography–tandem mass spectrometry (LC-MS/MS) assays are not broadly discussed in publications. Certain aspects of the development process are thus learned via experience. This article touches on a number of aspects that should be contemplated during method development for LC-MS/MS tests beyond sample preparation, chromatographic separation, and mass spectrometric detection. Utilization of factors intrinsic to LC-MS/MS, such as isotopically labeled internal standards and appraisal of transition ratios, engenders confidence in assay development and accelerates movement toward validation and testing.

**Development of a 25-Hydroxyvitamin D Liquid Chromatography–Tandem Mass Spectrometry Assay, Cleared by the Food and Drug Administration, via the De Novo Pathway** 553

Nicole V. Tolan

Despite great improvement in vitamin D assay standardization, inaccurate recoveries of 25(OH)D<sub>2</sub> remain for immunoassays, and many laboratory-developed LC-MS/MS methods do not separate out the 3-epimer interferents. Through the process of obtaining Food and Drug Administration (FDA) clearance, we learned that communication is key. Mass spectrometry–based assays raise different questions of safety and efficacy than the predicate immunoassays, with fewer risks due to increased accuracy. This process required improving our quality management system to support the development and registration of an in vitro diagnostic device. There are similar examples for a number of analytes, requiring expeditious entry of FDA-cleared LC-MS/MS methods into clinical laboratories.