



Better health through
laboratory medicine.

May 9, 2016

Food and Drug Administration
Division of Dockets Management (HFA-305)
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852.

Subj: Docket No. FDA-2016-N-0610

Dear Sir/Madam:

The American Association for Clinical Chemistry (AACC) appreciates the opportunity to provide comments to the Food and Drug Administration (FDA) related to its May 2, 2016 public workshop requesting information regarding the validation of liquid chromatography-mass spectrometry (LC-MS) based protein and peptide assays. We applaud the agency's willingness to enter into a public dialogue with the healthcare community on this important issue.

AACC is a global scientific and medical professional organization dedicated to clinical laboratory science and its application to healthcare. AACC brings together more than 50,000 clinical laboratory professionals, physicians, research scientists, and business leaders from around the world focused on clinical chemistry, molecular diagnostics, mass spectrometry, translational medicine, lab management, and other areas of laboratory science to advance healthcare collaboration, knowledge, expertise, and innovation.

Over the past few decades, ongoing innovation in clinical chemistry, mass spectrometry and separation sciences has played an indispensable role in advancing the field of laboratory medicine. This innovation has dramatically improved assay precision, accuracy and throughput, and the quality of test results and patient care. We agree with the FDA that analytical validation is a key element of this progress. Our comments explain how the current validation process works as well as provides details regarding existing safeguards in place to ensure that clinical laboratories provide quality, useful test results.

Regulatory and Accreditation Standards

Currently, all LC-MS based protein or peptide assays are regulated under the Clinical Laboratory Improvement Amendments (CLIA). These assays are categorized as high complexity assays, subject to stringent personnel, technical and clinical validation, quality control and proficiency testing requirements as well as regular inspections. In addition, many of these laboratories

participate in private sector accreditation programs, such as the College of American Pathologists and Joint Commission, as well as meet New York State regulatory requirements.¹ AACC supports the existing public-private partnership.

Tiered Classification of LC/MS Peptide/Protein Assays

Not all protein/peptide measuring systems employing mass spectrometry (including liquid chromatography or direct analysis) are equal. The broad spectrum of possible methodologies utilizing MS for protein and peptide analysis precludes the possibility of developing detailed guidance that could apply equally to all types of measuring systems. We therefore suggest that protein/peptide assays not be regulated as a single group, since the variety of analyses yields assays that differ widely with regards to the number of protein targets measured, type of quantitation, and post-analytical data processing.

A fundamental distinction within MS-based protein assays is the number of total analytes measured. Similar to MS-based assays for small molecules that measure a few or many analytes in a single run, protein and peptide assays differ in their degree of complexity. Further, multiplexed protein or peptide assays may be reported as independent measurements for clinical use or as individual components of a composite diagnostic index measurement. Some current MS-based assays perform direct measurements of intact proteins, while others use peptides as surrogate markers for protein quantification; these categories in turn require different analysis pipelines to determine accurate quantitation. Proteins and peptide assays may measure existing, well-established markers such as thyroglobulin, or they may be based on measuring novel biomarkers. Thanks to efforts undertaken by the clinical laboratory community, many novel protein and peptide markers are under development to serve patients' needs, and the possibility of developing future additional assays for novel markers with high analytical specificity is, in fact, a strength of mass spectrometry.

Despite these differences, we believe that workflow and characterization principles, particularly in the case of one or a small number of proteins, are consistent with all other quantitative techniques. The general principles of assay validation, as defined in existing CLSI guidance documents and standards and other references, share similar validation principles to non-LC/MS assays (including MALDI-MS based analysis). The agency's current thinking regarding validation of LC assays (e.g., LC-UV or LC-ECD assays), mass spectrometry assays (e.g.,

¹ Some examples of the existing guidelines that are applicable to protein or peptide based LC-MS assays include CLSI (Clinical and Laboratory Standards Institute) C62-A (*Liquid Chromatography-Mass Spectrometry Methods; Approved Guideline*), EP05-A3 (*Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition*), EP6-A (*Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*), EP17-A2 (*Protocols for Determining the Limits of Detection and Limits of Quantitation, Approved Guideline—Second Edition*), EP07-A2 (*Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition*), EP25-A (*Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*), and EP32-R (*Metrological Traceability and Its Implementation; A Report*). These documents have played critical roles in guiding the validation process of LC-MS assays.

newborn screening, immunosuppressants) and protein assays (e.g., immunoassays, electrophoresis) should apply equally to LC-MS assay of proteins and peptides regardless of the underlying technology.

Pre-analytical and analytical considerations

The pre-analytical phase of testing is an important area to consider during the validation process. This is where most laboratory errors occur. Controls for the pre-analytical phase for all clinical assays should include, but not be limited to, the preparation of the patient, blood draw process, sample collection method (sample type, tube collection, and protease inhibitor), storage conditions, transportation conditions, and analyte stability. The sample preparation phase should include, but not be limited to, proteinase selection, digestion conditions, protein or peptide capture antibody selection, and protein/peptide purification and enrichment process selection.

Lack of suitable reference materials is an ongoing concern not only for LC-MS methods, but also for many other clinical laboratory assays. The identification and selection of high purity reagents, calibrators, internal standards and other reference materials is a high priority for the laboratory. The quality of these products needs to be supported by all the relevant suppliers/manufacturers.

The LC-MS analytical phase considers additional factors such as column selection and HPLC and MS parameters selection. After samples are analyzed through LC and MS, data processing components such as calibration scheme, peak integration method, and data reporting should be considered. CLSI C62-A, Liquid Chromatography-Mass Spectrometry Methods; Approved Guideline, is a good reference for verification and validation of LC-MS assays prior to their implementation in the clinical laboratory. It is critical that the assay measuring system ensure that processing conditions affecting analytical performance are tightly controlled within the method as established by the assay developer or clinical laboratory. Validation should establish the procedure controls of these variables, separately or in aggregate, as appropriate, within the context of the overall assay performance assessment and specifications. While these factors represent important assay variables for LC-MS-based assays, they should be considered primarily within the existing framework of guidelines for assay validation rather than as an exhaustive, mandated list that may not adequately match all MS-based workflows for protein characterization.

Predicate devices

The issue of predicate devices is a major challenge for LC-MS regulatory submissions. In many cases, LC-MS methods exhibit analytical performance different from currently available methods. In some cases, there are no predicate devices. The predicate device requirements should be as flexible as possible, recognizing that in the case of certain measurands, the concept of a "predicate device" may not be applicable to this technology. In particular, it is very

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important to recognize that some inherent differences become apparent when comparing measured values between traditional immunoassays and LC-MS-based assays where specific proteoforms or proteolytic peptides are targeted with high analytical specificity.

Harmonization and Standardization

LC-MS has demonstrated superb consistency across different labs, different instrument models and different assays. Harmonization can be achieved by various means, including traceability to commutable samples or use of a central reference lab. Standardization can be achieved by reference methods procedures, certified reference material or traceable calibration materials.

Harmonization is very important, but it is also important to recognize that many current FDA-cleared clinical assays have well-documented deficiencies from the viewpoint of harmonization. While LC-MS has demonstrated a superior level of harmonization in many cases, we believe that any proposed FDA standards focused on harmonization should be consistent across all analytical principles of measurement and not uniquely focused on LC-MS.

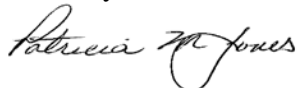
Platform consideration

Protein and peptide assays can be performed on various platforms, including LC-MS. The validation requirements for LC-MS assays for proteins and peptides should be similar to those for current IVD instruments/methods, e.g., as described in CLSI C62-A. While recognizing that some LC-MS specific considerations must be addressed, we believe that it is important that regulatory guidelines not place any additional burden on this particular technology relative to other established clinical laboratory analysis technologies just because of the potentially superior analytical capability of LC-MS.

Summary

In summary, we applaud the FDA's efforts to engage the laboratory community regarding issues pertaining to the validation of LC-MS based protein and peptide assays. Given the diversity of current and emerging LC-MS-based protein and peptide assays, there is no 'one best way' for regulating these tests. Fortunately, the current regulations and guidelines are sufficiently flexible to permit clinical laboratories to appropriately validate LC-MS assays. AACC looks forward to continuing to a dialogue with the FDA on this important issue. If you have any questions, please email Vince Stine, PhD, AACC Director of Government Affairs, at ystine@aacc.org.

Sincerely,



Patricia M. Jones, PhD, DABCC, FACB
President, AACC