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Mini-Review

Impact of VALID Act implementation on mass spectrometry-based clinical proteomic laboratory developed tests

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ABSTRACT

Mass spectrometry (MS)-based clinical proteomic Laboratory Developed Tests (LDTs) for the measurement of protein biomarkers related to endocrinology, cardiovascular disease, cancer, and Alzheimer's disease are gaining traction in clinical laboratories due to their value in supporting diagnostic and treatment decisions for patients. Under the current regulatory landscape, MS-based clinical proteomic LDTs are regulated by Clinical Laboratory Improvement Amendments (CLIA) under the auspices of the Centers for Medicaid and Medicare Services (CMS). However, should the Verifying Accurate Leading-Edge In Vitro Clinical Test Development (VALID) Act pass, it will grant the FDA greater authority to oversee diagnostic tests, including LDTs. This could impede clinical laboratories' ability to develop new MS-based proteomic LDTs to support existing and emerging patient care needs. Therefore, this review discusses the currently available MS-based proteomic LDTs and their current regulatory landscape in the context of the potential impacts imposed by the passage of the VALID Act.

1. Introduction

Proteins and peptides in biological specimens can serve as biomarkers for disease diagnosis and prognosis [1]. The history of using proteins for disease diagnosis dates back to 1827, when urinary albumin was noted to be an indicator of kidney disease. In 1845, Bence Jones protein, the first tumor biomarker, was identified as a diagnostic biomarker for multiple myeloma [2].

Protein biomarkers have conventionally been measured by immunoassay; however, due to the known limitations of immunoassays related to their poor specificity and selectivity, the adoption of mass spectrometry (MS)-based proteomic assays by clinical laboratories is increasing [3]. The primary focus of MS-based clinical proteomics in the clinical laboratory setting is the qualitative or quantitative measurement of proteins and peptides [4]. Compared to traditional immunoassays, MS-based proteomic methods can measure the intrinsic properties of multiple proteins within a single data acquisition, allowing for the measurement of analytes of interest with high specificity and selectivity. Additionally, the development, validation and modification of MS methods is relatively fast and cost-effective, offering the opportunity to expeditiously launch new assays on MS platforms in response to the changing needs of patient populations served by clinical laboratories. The passage of the Verifying Accurate Leading-Edge In Vitro Clinical Test Development (VALID IVCT) Act of 2021 (VALID Act) could have considerable unintended negative consequences on the ability of clinical laboratories to conduct existing and develop novel MS-based clinical proteomic assays.

The general workflows for MS analysis of proteins are shown in Fig. 1. There are two distinct analytical strategies for protein analysis using MS: intact protein analysis (commonly referred to as "top-down") and enzymatic proteolysis-aided analysis (known as "bottom-up"). Intact protein analysis typically entails the measurement of the mass-to-charge ratio (m/z) of the intact protein molecule using liquid chromatography (LC) coupled with high resolution MS. On the other hand, proteolysis-aided analysis requires enzymatic digestion to process the intact protein into smaller peptides, which are used as surrogates for protein identification and quantification. Enrichment or fractionation can be conducted to overcome some of the challenges posed by the matrix complexity of the sample.

Abbreviations: LDT, Laboratory developed test; VALID Act, Verifying Accurate Leading-Edge In Vitro Clinical Test Development Act; CLIA, Clinical Laboratory Improvement Amendments; MS, Mass spectrometry; LC, Liquid chromatography.

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2. Current regulatory landscape of MS proteomic LDTs

According to the Food and Drug Administration (FDA)'s definition [5], an LDT is a laboratory test that is developed and used within a single laboratory. MS-based proteomic LDTs must undergo thorough systematic development and validation before being added to a clinical laboratory's test menu. The quality requirements for MS assays to achieve acceptable measurement uncertainty are rigid, and these assays must be conducted within the same quality assurance framework required for other tests used in clinical laboratories [3]. Currently, no LC–MS-based proteomic LDTs have FDA approval [7].

Detailed guidelines for the development and validation of proteomic LDTs are provided in the recently-published Clinical and Laboratory Standards Institute (CLSI) C64 "Quantitative Measurement of Proteins and Peptides by Mass Spectrometry" guidance document [8]. Additional guidelines for MS test development and validation can be found in the CLSI C62 "Liquid Chromatography–Mass Spectrometry Methods" [9] and C50 "Mass Spectrometry in the Clinical Laboratory" guidance documents [10], as well as the FDA document "Bioanalytical Method Validation Guidance for Industry" [11]. Of these CLSI documents, C64 is the only one that specifically addresses MS-based proteomic assays. An overview of the general principles of MS-based proteomic LDT development and validation is provided in the following section [8].

3. Development and validation of LC–MS proteomic LDTs

The development and validation process aims to comprehensively evaluate a test to ensure the accuracy and precision of the test results, and to minimize the risk of a clinically significant measurement error. Before conducting a comprehensive validation, the test developer should define the acceptance criteria for each validation component based on the property and intended clinical utility of the test. Compared to traditional immunoassays, MS-based proteomic test workflows are more complicated, and total automation is not yet available [3].

Assay development minimally consists of the test materials, an internal standard, MS data acquisition parameters, an LC separation method, sample preparation, calibration materials, and quality control materials [12]. The LC–MS-based clinical proteomic assay development process has three stages: feasibility and planning, empirical development and optimization, and pre-validation evaluation. During the feasibility and planning step, the clinical utility, potential risk, regulatory, practical and analytical considerations should be taken into account. The identity of the measurand (the quantity intended to be measured, as defined by international convention) and the choice of surrogate peptides (if a bottom-up workflow is used) are important considerations during the feasibility and planning stage. After defining the measurand, the test developer should follow prescriptive guidance for LC–MS assay development and optimization, such as the framework provided by the CLSI guidance documents.

Preliminary evaluation (i.e., pre-validation) should be performed to evaluate the initial version of the assay before expending resources to conduct a comprehensive validation. In this phase, the test developer should evaluate the assay using the intended specimen type(s), and should include samples from healthy and diseased individuals. Accuracy, inter- and intra-run precision, sample preparation-related analyte recovery, sample stability, carryover, and selectivity should be assessed during the pre-validation phase. It is worth noting that even though the development process is described here as a sequential process, the development process is often not linear, but rather iterative, and in practice, there is overlap between different development stages.

Several parameters should be evaluated during the validation process. The first parameter is the total, or within-laboratory imprecision, which should encompass the common sources of variation associated with running the test using the same instrument in a given laboratory. It is generally assumed that the variation, associated with repeated analysis, follows a normal, or Gaussian, distribution. If multiple test operators and instruments within a single laboratory will be involved in running the test, which is often the case for LC–MS-based proteomic assays, imprecision and inter-operator and inter-instrument agreement should be assessed as well.

The lower limit of the measuring interval (LLMI) must be defined during the validation process. A 20 % coefficient of variation (CV) of imprecision is typically acceptable for most LC–MS/MS assays; however, when the clinical decision limit is close to the LLMI, acceptable imprecision of clinically relevant values should also be considered. Linearity and extended measurement intervals should also be evaluated. Extended measurement intervals are assessed by diluting the specimen to decrease the concentration of the measurand to be within the measurable range,

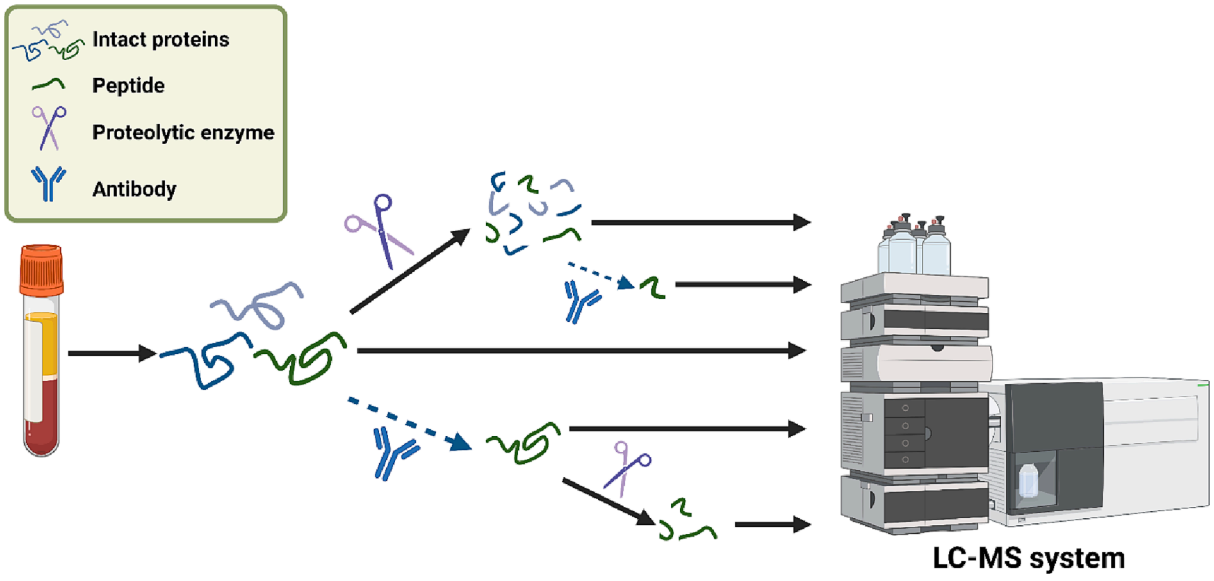

Fig. 1. General workflow for the LC–MS analysis of proteins in the clinical laboratory setting. The proteins in the biological specimen are submitted to either enzymatic proteolysis-aided or intact protein analysis. Given the complexity of the clinical specimen and the endogenous abundance of the target protein, antibody-based enrichment may be required. This enrichment can be integrated into different stages of the sample preparation workflow, either at the protein or peptide level. Figure created with BioRender.com.

and the maximum dilution factor is determined to ensure acceptable imprecision of the results from the analysis of the diluted samples. Additionally, measurand and reagent stability should be assessed [13]. Interference from antibody cross-reactivity is a common challenge for immunoassays, and interference due to co-eluting analytes with similar chemical properties can also impact the performance of MS-based assays; thus, specificity is an essential part of the assay validation process.

Selected reaction monitoring (SRM), also referred to as multiple reaction monitoring (MRM), is a commonly used targeted MS data acquisition method that is often employed using a triple quadrupole (QqQ) mass spectrometer. SRM methods involve monitoring a precursor ion and one or several fragment ions derived from the precursor ion to enable selective and specific measurement of the analyte(s) of interest [14]. During the development of LDTs incorporating SRM, the optimization of transition ion ratios (defined as the ratio of the peak height or peak area intensity of the quantifier ion to that of the qualifier ion) should be completed to minimize interference. MS-based assays present a unique challenge in the form of ionization suppression, which can sometimes be significant due to the presence of co-eluting species. Carryover should also be evaluated during the method development process using low, medium, and high measurand concentrations.

The validation of the aforementioned parameters should be conducted in a workflow- and matrix-dependent manner. If alternative workflows or matrices are required for the analysis of the measurand, additional validation should be undertaken.

4. Representative LC–MS proteomic LDTs

Currently available MS-based clinical proteomic LDTs measure a range of proteins with functions related to endocrinology, microbiology, cancer, and Alzheimer's disease (Table 1). These tests are used in Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories to support patient care. Below, we provide an overview of two of these MS-based clinical proteomic LDTs that are integral to patient care.

4.1. Thyroglobulin

Thyroglobulin is a tumor marker that can be monitored to guide thyroid cancer treatment and evaluate recurrence. LC–MS/MS measurement of thyroglobulin can report accurate thyroglobulin concentrations in spite of the presence of anti-thyroglobulin antibodies in approximately 30 % of thyroid cancer patients [15]. These autoantibodies to thyroglobulin often cause assay interference and lead to falsely low thyroglobulin concentrations when measured using immunoassays [16]. Thyroglobulin is not the only clinically-relevant protein whose accurate measurement is challenged by the presence of auto-antibodies. Other examples include prolactin and insulin [17]. The limitations of immunoassays make MS a valuable analytical method for the measurement of such proteins, offering superior specificity and selectivity.

LC–MS/MS-based thyroglobulin LDTs are in use at several laboratories, including ARUP Laboratories, Laboratory Corporation of America Holdings (LabCorp), Mayo Clinic, and the University of Washington [18]. These LDTs employ various sample preparation techniques, some of which include an enrichment step prior to proteolysis. The surrogate thyroglobulin tryptic peptides are then used for quantification. A pilot study [18] showed that the harmonization of LC–MS/MS thyroglobulin LDTs across these four laboratories is as good as or better than that of thyroglobulin immunoassays. This is supported by the lower CV of the LC–MS/MS-based assay (11.6 %) compared to the immunoassay (21.9 %) when evaluating the agreement between the average measurand concentration across the four laboratories and the concentration reported by each individual laboratory. Following well-established guidelines for assay development and validation will result in reliable inter-laboratory thyroglobulin concentrations measured by LC–MS/MS LDTs, even when they utilize different sample preparation methodologies, internal standards, calibration methods, and surrogate peptides.

Table 1
Representative protein biomarkers in LDTs and their clinical applications.

Protein biomarker	Clinical Applications
Thyroglobulin	Thyroid cancer [15]
Parathyroid hormone	Calcium metabolism disorders [28]
Beta-Amyloid 42/40 Ratio	Alzheimer's disease [29]
Apolipoprotein	Cardiovascular disease [18]; Alzheimer's disease [19]
Insulin-like growth factor 1	Growth disorder [30,31]
Hemoglobin	Diabetes mellitus (DM) [32]
Insulin	DM differential diagnosis [33]
Transthyretin	Transthyretin-associated familial amyloidosis [34]

4.2. Apolipoproteins

Apolipoproteins are other examples of clinically relevant proteins whose measurement by LC–MS/MS fulfills a previously unmet clinical need. Apolipoproteins are essential for lipid metabolism, and their isoforms have diverse functions. Apolipoproteins (Apo) A and B mainly bind to high-density lipoprotein (HDL) and low-density lipoprotein (LDL), respectively, and they are often tested separately or simultaneously for the evaluation of cardiovascular diseases. Apo C and E have functions related to triglyceride metabolism [19]. In addition, Apo E is a major brain apolipoprotein that carries cholesterol and can serve as a biomarker for Alzheimer's disease [20]. FDA-approved immunoassays are not available for these apolipoprotein isoforms.

The LC–MS/MS analysis of serum apolipoproteins allows the quantification of multiple apolipoproteins, including Apo A-I, B, C-I, C-II, C-III, and E, and the phenotyping of apolipoprotein E using a single LC–MS/MS method [21]. As the concentration of apolipoproteins in serum is relatively high, an enrichment step is not required during sample preparation. This multiplexed LC–MS/MS-based apolipoprotein assay provides insight into the composition of apolipoprotein isoforms, enabling the differential diagnosis of dyslipidemia types and personalized treatment options for individual patients.

Another clinical application of proteomic-based LDTs for the measurement of apolipoproteins is the determination of cholesterol efflux capacity for the prediction of coronary artery disease [22]. In this LC–MS/MS assay, 21 lipoprotein-related proteins are quantified, including Apo A-I, C-I, C-II, C-III, and C-IV, to establish a cholesterol efflux capacity prediction (pCE) model. A coronary artery disease prediction (pCAD) algorithm was derived when evaluating this pCE model in a case-control study, using specimens from coronary artery disease patients and healthy individuals. Using the pCAD as a classifier for patients versus healthy controls, the area under the ROC curve was 0.73. This study demonstrates that LC–MS/MS assays, with their multiplexing capacity, offer an opportunity to measure apolipoproteins with high specificity and selectivity.

4.3. Precision oncology-related proteins

Novel opportunities for MS-based clinical proteomic LDTs exist within the realm of precision oncology [23–26]. Many cancer patients do not respond to prescribed targeted therapies based on the genomic profiles of their tumors; however, MS-based clinical proteomic LDTs could be developed, validated, and deployed to address this unmet and urgent clinical need, providing accurate protein measurements to predict oncology treatment response. Unfortunately, these innovative efforts would largely be stymied by the implementation of the VALID Act.

5. FDA oversight of LDTs via the VALID Act

Currently, all MS-based clinical proteomic assays are LDTs. These LDTs, including MS-based clinical proteomic LDTs, are subject to CLIA regulations under the auspices of the Centers for Medicare and Medicaid Services (CMS).

The VALID Act would provide the FDA with greater authority to regulate diagnostic tests, and it would create a risk-based framework for regulating LDTs, similar to the approaches the FDA takes toward other medical devices. A new classification of in vitro clinical tests (IVCTs) is proposed within the VALID Act, which would include LDTs and in vitro diagnostic (IVD) tests currently regulated by the FDA. If the VALID Act is implemented, it is possible that existing LDTs could be "grandfathered" (i.e., exempted from FDA review) into the new regulatory framework, although the details regarding this option are unclear. New IVCTs would be regulated under a risk-based framework that categorizes IVCTs as high-, moderate-, and low-risk. High-risk IVCTs would require premarket review and approval from the FDA, while low-risk IVCTs could be launched after passing technology certification, which would allow test developers to be exempted from FDA approval for each new test after approval for one representative test utilizing the same technology [6]. IVCTs within the high- or moderate-risk categories would be subjected to the FDA's premarket review and approval process, which could take up to eight months.

Implementation of the VALID Act would significantly impede the ability of clinical laboratories to quickly and effectively respond to new patient care needs by providing solutions involving accurate, sensitive, and selective protein measurement. Whereas the development, validation, and FDA approval process for a novel immunoassay could take at least two years, the development and validation process for LC–MS/MS-based clinical proteomic LDTs can be completed within a few weeks to a couple of months. If clinical proteomic LDTs are subjected to FDA oversight as proposed in the VALID Act, the review and approval process would likely take the same amount of time as the lengthy process for novel immunoassays.

6. Conclusions

Currently available MS-based clinical proteomic LDTs demonstrate their versatility and value within the context of patient care. The new regulatory landscape proposed in the VALID Act would require clinical labs, many of which are facing unprecedented resource constraints [27], to become involved in the lengthy and often arduous FDA regulatory filing and reviewing process, which could delay patients' access to new LDTs. Of greater concern is the possibility that existing LDTs could be removed from labs' test menus if the lab lacks the resources to support FDA regulatory filing. This situation could be exacerbated for MS-based clinical proteomic LDTs, given that the development and validation of these tests is costlier than other more routine clinical laboratory assays due to their complexity. The FDA approval process for medical devices and diagnostic tests has historically been an endeavor largely undertaken by for-profit entities.

The development and validation processes for LDTs are integral components of the training of Laboratory Medicine and Pathology residents, fellows, and students, many of whom pursue careers as medical directors of clinical laboratories. A substantial portion of their training occurs within clinical laboratories at academic medical centers. If clinical laboratories are forced to pivot away from the development and validation of new LDTs due to the significant burden imposed by the new regulatory landscape associated with the VALID Act, these critically important educational activities will cease to exist.

To counter the viewpoint that FDA involvement in the regulation of LDTs could enhance patient safety, it is important to note that the FDA's regulatory and review process would likely still be based on the development and validation guidelines that clinical laboratories follow within the current regulatory landscape under CLIA. This is a robust and well-established system. Instead of deploying resources to significantly increase the regulatory compliance burden that clinical labs would be faced with under the VALID Act, consideration should be given to allocating resources to other efforts, such as inter-laboratory harmonization of LDTs.

In conclusion, the implementation of the VALID Act would likely impede the future development of MS-based clinical proteomic LDTs, which are an important diagnostic tool for patients. This would impose strict, costly, and largely redundant regulations on clinical labs, compromising their ability to offer these tests, and reducing the quality of patient care.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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