*Saudi J. Med. Pub. Health* **Vol. 2,** No. 2, pp 757-765 (2025)

**Saudi Journal of Medicine and Public Health**

https://saudijmph.com/index.php/pub

https://doi.org/10.64483/jmph-196

# Mass Spectrometry in the Clinical Laboratory: Beyond Toxicology to Endocrinology and Microbiology

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## Abstract

**Background:** The clinical laboratory has witnessed a paradigm shift with the introduction of mass spectrometry (MS) into its activities, extending its role way beyond the traditional field of toxicology.
**Aim:** This review comprehensively discusses the revolutionizing impact of tandem mass spectrometry (MS/MS) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS on the diagnosis and management of human disease, particularly in endocrinology and microbiology.
**Methods:** Synthesis of concepts, applications, and relative advantages of these MS methods from the current literature between 2015 and 2024 was reviewed.
**Results:** Liquid chromatography-tandem mass spectrometry (LC-MS/MS) emerged as the gold standard in endocrinology with improved specificity for steroid, thyroid hormone, and vitamin measurement, thereby enabling accurate diagnosis of complex disorders. Meanwhile, MALDI-TOF MS has revolutionized clinical microbiology by providing quick, accurate, and cost-effective microbial identification, with an appreciable shortening of the time to effective antimicrobial therapy. Further, MS/MS remains the basis of newborn screening for congenital errors of metabolism. Despite challenges like standardization and cost, newer directions like mass spectrometry imaging and ambient ionization techniques promise further advancements.
**Conclusion:** The findings verify that MS/MS and MALDI-TOF MS lead the path of the modern clinical laboratory's commitment to deliver precise, individualized, and prompt patient treatment.

**Keywords:** Mass Spectrometry, Clinical Diagnostics, Endocrinology, Microbiology, Newborn Screening.

## 1. Introduction

For decades, the clinical lab has relied chiefly on automated immunoassays for the measurement of hormones and phenotypic methods for microbial characterization. While these techniques have been the bread and butter of diagnostic medicine, they are beset with their shortcomings, like cross-reactivity, poor specificity at low concentrations, and a protracted time-to-result, particularly in microbiology (Grebe & Singh, 2011). The entry of mass spectrometry into the clinic, which initially was for drug screening and analysis of small molecules, promised to overcome these limitations. Mass spectrometry yields the ultimate analytical specificity by separating and detecting ions based on their mass-to-charge ratio (m/z), necessarily distinguishing from the inferential process of immunoassays (Annesley, 2009).

Two MS platforms have contributed to such diagnostic transformation. The first is tandem mass spectrometry (MS/MS), often combined with liquid or gas chromatography (LC or GC). LC-MS/MS is effective in quantifying a wide range of analytes in complex biological matrices and will thus lend itself to measuring low-abundance, structurally similar molecules like steroids and thyroid hormones (Rauh, 2012). The second is matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS, which is excellent at measuring intact proteins and biomolecules rapidly, a feature ideally suited to obtaining distinctive spectral "fingerprints" of microorganisms (Patel, 2015).

The globalization of MS from specialized reference labs to hospital-based clinical laboratories has been a characteristic trend over the last decade. This review will examine this growth in three principal diagnostic areas. First, it will outline how LC-MS/MS is replacing immunoassays in endocrinology, with specific examples of its influence on the diagnosis of disorders such as Cushing's syndrome, congenital adrenal hyperplasia, and hypothyroidism. Second, it will chronicle the disruptive effect of MALDI-TOF MS in clinical microbiology, highlighting how it has contributed to streamlining workflows for the identification of bacteria and fungi and in its novel

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Receive Date: 28 September 2025, Revise Date: 28 October 2025, Accept Date: 31 October 2025

functions for the detection of antimicrobial resistance. Third, it will reaffirm the value of MS/MS in screening for inborn errors of metabolism, a public health success story. By incorporating contemporary scientific literature (2015-2024), this review tries to provide a comprehensive picture of how these mass spectrometry technologies are enhancing diagnostic accuracy, maximizing operational efficacy, and ultimately paving the way for more customized and efficient patient management.

## Fundamentals of the Critical Mass Spectrometry Platforms

### Tandem Mass Spectrometry (LC-MS/MS and GC-MS/MS)

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) is a hyphenated analytical method that integrates the physical separation ability of liquid chromatography with the ultra-high detection specificity of mass spectrometry. Sample preparation is the beginning of the process and takes a critical position in the removal of interfering substances and the concentration of the analyte of interest. The extracted sample is introduced into the LC system, where compounds are resolved by virtue of the chemical affinity they possess for the stationary phase of the chromatographic column (Ho et al., 2003). Chromatographic separation serves to resolve analytes over a period of time, with minimal ion suppression and matrix effect in the mass spectrometer.

Following LC, the eluent is channeled to the mass spectrometer, which operates in three fundamental modes. First, ionization: most clinical LC-MS/MS employs electrospray ionization (ESI), which gently carries analytes from the liquid to the gas phase as charged ions (Keevil, 2016). Second, mass selection: the first quadrupole mass analyzer (Q1) is a filter that allows only the precursor ion of a specific m/z to pass. Third, fragmentation and detection: the selected ions are broken up in a collision cell (q2) with an inert gas, and the resulting product ions are detected using a second quadrupole (Q3). This two-step mass analysis produces high specificity because a transition between a specific precursor ion and a specific product ion is being detected for each analyte (Thomas et al., 2022). Its ability to monitor multiple such transitions simultaneously in a single run—a process known as multiple reaction monitoring (MRM)—is what makes LC-MS/MS so powerful for multiplexed panels, such as those for newborn screening and steroid profiling.

Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS), while of historical importance, is now in most situations limited to volatile compounds or the readily derivatizable compounds to facilitate increased volatility and thermal stability. It remains a gold standard for some toxicology analyses as well as analysis of organic acids for metabolic disorder diagnosis (Rashed, 2001).

### Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) MS

MALDI-TOF MS is based on a qualitatively different mode of operation than LC-MS/MS. It is designed for the rapid analysis of intact biomolecules, primarily proteins. The sample (e.g., a bacterial colony) is mixed with a chemical matrix and deposited as spots onto a target plate. The matrix crystallizes with the analyte, and when pulsed with a laser, it releases its energy, causing desorption and ionization of the analyte with minimal fragmentation (Singhal et al., 2015).

These ions are then accelerated into a time-of-flight (TOF) mass analyzer through an electric field. The ions are given equal kinetic energy, and hence velocity is inversely proportional to the square root of m/z. Faster-moving light ions reach the detector before heavier ions. By measuring the time it takes for every ion to traverse the flight tube length, the instrument is capable of determining its m/z, resulting in a mass spectrum that is a unique protein fingerprint of the microorganism (Angeletti, 2017). This spectrum, with a high predominance of the very highly abundant ribosomal proteins, is then compared against a large reference spectrum database for identification. The entire process, from sample preparation to identification, can be done within minutes, as opposed to 24-48 hours from conventional biochemical methods (Chen et al., 2021).

## The Established Foundation: MS in Toxicology

Clinical application of mass spectrometry was the pioneer in the toxicology discipline. Immunoassays for drug-of-abuse are notoriously prone to false positives (cross-reactivity) and false negatives (insensitivity), so confirmation is required (Jannetto & Fitzgerald, 2016). LC-MS/MS and GC-MS emerged as the standards against which the confirmation should be done, and provide absolute proof of the presence and quantity of a specific drug or metabolite.

LC-MS/MS panels can completely screen and confirm scores of analytes like opioids, benzodiazepines, stimulants, and synthetic cannabinoids from a single sample (Maurer & Meyer, 2016). This capability is crucial for surveillance of compliance with pain management, post-mortem toxicology, and confirming suspected cases of overdose. The specificity of MS allows for the differentiation of parent drugs and their metabolites and other structurally related compounds that immunoassays cannot distinguish from one another, such as distinguishing between fentanyl and its various analogs (Palmquist et al., 2023). The success of MS in normalizing toxicological screening and improving its accuracy provided the proof-of-concept and operating information necessary for scaling up MS to other, more complex, laboratory applications. Figure 1 shows the comparative analytical workflow: LC-MS/MS vs. MALDI-TOF MS.

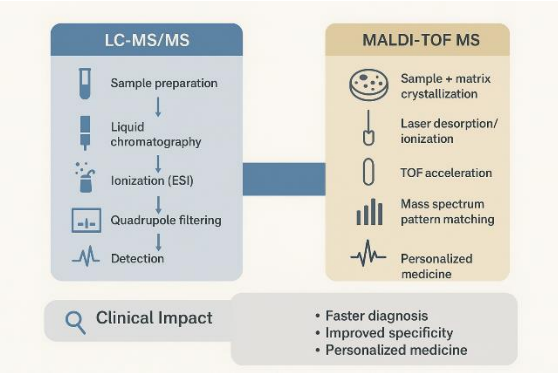

**Figure 1: Comparative Analytical Workflow: LC-MS/MS vs. MALDI-TOF MS**

## The Endocrine Revolution: LC-MS/MS in Hormone and Vitamin Analysis

The limitations of immunoassays are greatest in endocrinology, where many hormones have low concentrations and are present in the presence of structurally related analogs or binding proteins. LC-MS/MS is now the ultimate method for overcoming these hurdles, providing a new level of diagnostic precision (Table 1).

### Steroid Hormone Profiling

The steroid analysis is an exemplar for the benefit of LC-MS/MS over the traditional immunoassays, which are plagued by severe inaccuracies for important hormones like cortisol, aldosterone, testosterone, and vitamin D (Taylor et al., 2015). The virtue of LC-MS/MS is that it can quantify multiple steroids from a single sample simultaneously, reporting a snapshot of metabolism that is highly useful in diagnosing endocrine disorders. This capability is revolutionary across many of the major disciplines. For diagnostic purposes for Cushing's syndrome and congenital adrenal hyperplasia (CAH), LC-MS/MS provides a level of specificity unavailable with immunoassays. For Cushing's, it makes possible accurate measurement of cortisol during dynamic testing and late-night saliva without the interference of synthetic steroids or cross-reactivity with cortisone, the typical shortcoming of immunoassays (Eisenhofer et al., 2017).

For CAH, multiplexed panels assaying 17-hydroxyprogesterone, androstenedione, and 21-deoxycortisol in combination yield a far more reliable diagnostic and monitoring test. It is a vast improvement from the solitary 17-OHP immunoassay measurement, whose excessive cross-reactivity with other fetal adrenal zone steroids produces an unacceptably high rate of false positives, particularly in preterm infants (Janzen et al., 2007). Similarly, in andrology and reproductive endocrinology, LC-MS/MS is the procedure of choice now. Immunoassays lack significantly the sensitivity required to quantify the low levels of testosterone found in women, children, and hypogonadal men, but LC-MS/MS quantifies the hormone with precision throughout the entire range of physiological concentrations (Rowen et al., 2020). This analytical ability is also crucial for the simultaneous analysis of estradiol and estrone in fertility treatment planning and hormone-sensitive endocrine cancers, where precise hormonal differentiation is essential to good clinical decision-making.

### Thyroid Function Testing

While thyroid-stimulating hormone (TSH) immunoassays are trustworthy, the determination of thyroid hormones (thyroxine, T4; triiodothyronine, T3) is potentially compromised by autoantibodies, binding protein derangement, and metabolite interference. LC-MS/MS provides for direct quantitation of the free hormone fraction free from such interference (Kushchayeva et al., 2019). This is particularly useful in complex cases, e.g., pregnancy, critical illness, or heterophilic antibodies, where standard immunoassays are unreliable.

### Analysis for Vitamin D Metabolites

The "Vitamin D" status can be ascertained by measurement of 25-hydroxyvitamin D [25(OH)D], which is the major circulating metabolite. Immunoassays of 25(OH)D are marred by irregular cross-reactivity with its two forms (D2 and D3) and with other metabolites and therefore generate significant inaccuracies and unsatisfactory inter-laboratory concordance (Lai et al., 2012). LC-MS/MS is the method of choice because it can quantify individually 25(OH)D2 and 25(OH)D3, providing a true total concentration of 25(OH)D and revealing vitamin D2 supplementation, underestimated by immunoassays (Shah et al., 2016).

## The Microbiological Transformation: MALDI-TOF MS in Pathogen Identification

The introduction of MALDI-TOF MS to the clinical microbiology laboratory has been the most important change in the specialty over the past 20 years, having a dramatic speedup and improvement in the accuracy of microbial identification.

### Identification of Bacteria and Yeast

The conventional process of identification of bacteria includes Gram staining and culture on selective media followed by a series of biochemical tests, a process that can take 24-48 hours or more. MALDI-TOF MS shatters this paradigm through the ability to identify microorganisms directly from one agar plate colony in less than 5 minutes (Chen et al., 2021; Asiri et al., 2025). MALDI-TOF MS has been consistently shown to achieve species-level identification at levels of accuracy comparable to or superior to current standard techniques in a broad majority of clinically relevant bacteria and yeasts, such as Candida species (Clark et al., 2013). This concomitant identification has a direct impact on patient care through the initiation of earlier targeted antimicrobial therapy.

**Table 1: Comparative Analysis of Immunoassay vs. LC-MS/MS for Key Endocrine Analytes**

| Analytic | Clinical Application | Limitations of Immunoassay | Advantages of LC-MS/MS |
|---|---|---|---|
| **Testosterone** | Diagnosis of male hypogonadism; evaluation of female androgen excess. | Poor sensitivity and specificity at low concentrations (e.g., in women and children). | High sensitivity and specificity across the entire physiological range; no cross-reactivity with other steroids (Peitzsch et al., 2015). |
| **Cortisol** | Diagnosis of Cushing's syndrome; adrenal insufficiency. | Cross-reactivity with synthetic steroids (e.g., prednisolone) and cortisol metabolites (e.g., cortisone). | High specificity for cortisol; accurate measurement in dynamic testing and in complex matrices like saliva (Wang et al., 2020). |
| **Vitamin D [25(OH)D]** | Assessment of nutritional vitamin D status. | Variable cross-reactivity between D2 and D3; inability to distinguish forms; matrix effects. | Specific and separate quantification of D2 and D3; considered the reference method; superior accuracy (Lai et al., 2012). |
| **Aldosterone** | Diagnosis of primary aldosteronism. | Low specificity; susceptibility to interference; poor performance in low-renin conditions. | High specificity and sensitivity; enables accurate calculation of aldosterone-to-renin ratio (ARR) (Fuss et al., 2021). |
| **17-Hydroxyprogesterone** | Newborn screening and diagnosis of CAH. | High false-positive rate due to cross-reactivity with other steroids from the fetal adrenal zone. | Specific measurement; reduced false positives; can be multiplexed with other steroid precursors (e.g., 21-deoxycortisol) (Janzen et al., 2007). |

### Direct Specimen Analysis and Bloodstream Infections

One of the most impactful applications is the direct analysis of positive blood culture bottles. Instead of subculturing the bottle and waiting an additional 24 hours for colonies to develop, a direct sample can be taken from the positive broth, cleaned to remove blood cells and proteins, and analyzed using MALDI-TOF MS. This can provide a microbial identification 24-48 hours ahead of conventional practice (Hu et al., 2019). In sepsis, where every hour of delay contributes to increased mortality, this acceleration is particularly significant (Timbrook et al., 2016). While the sensitivity can be slightly lower than from purely cultured organisms, algorithms have been optimized to provide reliable identification for the most common bloodstream pathogens, such as Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus (Verroken et al., 2015).

### Antimicrobial Resistance (AMR) Detection

A recent limitation of traditional MALDI-TOF MS is that it is not able to routinely deliver antimicrobial susceptibility predictions directly from the mass spectrum. However, innovative solutions are being set to cover this gap. One of these approaches includes direct detection of documented resistance mechanisms. For example, the MBT STAR-BL assay (Bruker Daltonics) is employed for the detection of hydrolysis of a β-lactam antibiotic by β-lactamase-producing bacteria by observing the mass shift between the intact drug molecule and its hydrolyzed derivative (Ota et al., 2019). This can provide proof for carbapenemase production in Enterobacterales within hours. The other research-oriented approaches are analyzing spectral peaks that correlate with resistance, e.g., methicillin resistance in Staphylococcus aureus (MRSA) or vancomycin resistance in Enterococci (VRE), although these are not yet well-established practices within the clinic (Florio et al., 2020).

### Broadening the Horizon: MS/MS in Newborn Screening and Inborn Errors of Metabolism

Newborn screening (NBS) is among the most successful applications of mass spectrometry in public health. The application of MS/MS in NBS programs worldwide has enabled the detection of over 50 analytes from a single 3.2 mm dried blood spot (DBS), with dozens of potentially life-threatening inborn errors of metabolism (IEM) detected early before the appearance of symptoms (Therrell et al., 2015).

The MS/MS multiplexed feature allows for the detection of aminoacidopathies (such as phenylketonuria, maple syrup urine disease), organic acidemias (such as propionic acidemia, methylmalonic acidemia), and fatty acid oxidation disorders (such as medium-chain acyl-CoA dehydrogenase deficiency) in a single analysis run

(Chace et al., 2002). NBS early diagnosis allows for prompt medical or dietary treatment, preventing severe intellectual disability, metabolic crises, and death. The continuous optimization of MS/MS technology expanded NBS panel sizes and maximized screening positive predictive value, reducing false positives and distress to parents (Schmidt et al., 2012). Furthermore, LC-MS/MS is used for second-tier analysis of NBS results that are indeterminate and monitoring patients with known diagnoses, quantifying specific biomarkers to validate therapy efficacy (McHugh et al., 2011).

## Comparative Strengths and Integration into Clinical Workflow

The widespread acceptance of MS/MS and MALDI-TOF MS in today's clinical laboratory is fueled by a range of very strong benefits that directly address the limitations of traditional diagnostic methods. First among these is their unmatched specificity and sensitivity. LC-MS/MS accomplishes this through the combination of chromatographic separation and selective monitoring of unique mass transitions, allowing it to unambiguously distinguish between isobaric compounds that co-elute, an impossibility for cross-reactivity-vulnerable immunoassays. Similarly, MALDI-TOF MS identifies microorganisms based on a complex protein fingerprint, with a discrimination capability far superior to that of a restricted panel of biochemical reactions. Another key advantage is multiplexing ability; multiple tens of steroids, drugs, or metabolites are analyzed in one LC-MS/MS analysis from a minute sample volume, optimizing analytical productivity and conserving precious patient specimens, as opposed to the typically single-analyte concentration of automated immunoassays.

For microbiology, its short turnaround time is the hallmark feature of MALDI-TOF MS, cutting microbial identification to minutes instead of days and enabling direct facilitation of earlier, more targeted antimicrobial treatment, enhancing patient outcomes and supporting antimicrobial stewardship programs (Timbrook et al., 2016). Finally, the lack of specific reagent dependence offers a specific strategic flexibility. Since MS methods are based on physicochemical properties rather than on antibody-antigen interactions, laboratories can develop and validate in-house tests ("laboratory developed tests" or LDTs) for novel biomarkers independent of the availability or cost of commercial kits, thus accelerating the translation of novel diagnostic discoveries into practice (Grebe & Singh, 2011).

In spite of these robust strengths, integration of mass spectrometry is not free from serious challenges that deter its adoption. The substantial capital outlay for hardware and the ongoing requirement for highly skilled technical and scientific staff represent major economic and operational barriers to most laboratories (Thomas et al., 2022). Also, method development, validation, and troubleshooting activities are intrinsically complex and time-consuming compared to the relative simplicity of applying a standardized immunoassay. Compounding these issues is an ongoing lack of full platform-to-platform and laboratory-to-laboratory standardization. Sample preparation, instrument, and data processing variability can lead to inter-laboratory variability and affect the uniformity of patient results. Even though organizations like the Clinical and Laboratory Standards Institute (CLSI) are working diligently to address this challenge by providing guidelines for method harmonization and validation, achieving complete standardization is a continuous endeavor that is paramount to the future of clinical mass spectrometry (Stanczyk & Clarke, 2010). Table 2 & Figure 2 summarize the primary clinical applications of MS/MS and MALDI-TOF MS.

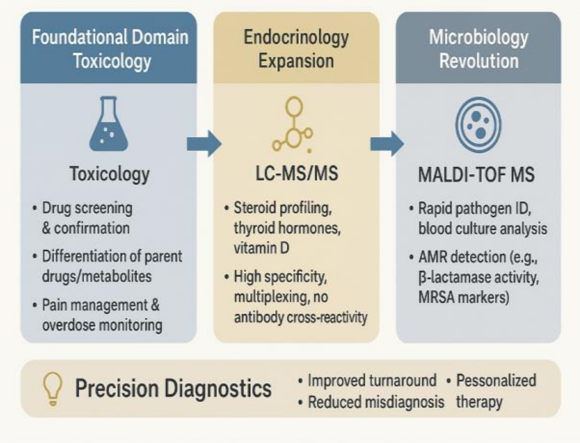

**Figure 2: The Expanding Clinical Applications of Mass Spectrometry**

## Challenges and Future Perspectives

The impact of mass spectrometry on the clinical laboratory is undeniable in its dramatic nature, but the discipline continues to evolve at a rapid pace, navigating its way through a landscape of continued challenge while embracing a cosmos of hope-filled technological frontiers in tandem. Integration of these evolving platforms is not without difficulty; however, that must be addressed methodically if their ultimate promise in day-to-day patient care is to be realized.

One of the biggest challenges is standardization and harmonization of methods in different laboratories. Use of different instruments, sample preparation protocols, and process algorithms may result in high inter-laboratory variability, leading to difficulty in interpreting the findings and in following up the patients in different healthcare systems. Thus, concerted effort by international organizations to create reference methods, certified reference materials, and harmonized best practices is crucial to ensure that patient results are comparable and consistent anywhere (Stanczyk & Clarke, 2010).

**Table 2: Summary of Primary Clinical Applications of MS/MS and MALDI-TOF MS**

| Platform | Primary Clinical Domain | Key Applications | Impact on Patient Care |
|---|---|---|---|
| **LC-MS/MS** | **Endocrinology** | Steroid profiling (testosterone, cortisol, aldosterone), Vitamin D quantification, Thyroid hormone testing, Catecholamine analysis. | Enables precise diagnosis of complex endocrine disorders; reduces misdiagnosis from immunoassay interference; guides personalized hormone therapy (Rauh, 2012). |
| **LC-MS/MS** | **Toxicology & TDM** | Comprehensive drug screening/confirmation, Quantification of chemotherapeutic agents, immunosuppressants, and antidepressants. | Provides definitive proof of substance use; guides dosing for drugs with a narrow therapeutic index; improves safety and efficacy (Jannetto & Fitzgerald, 2016). |
| **LC-MS/MS / MS/MS** | **Inborn Errors of Metabolism** | Newborn screening from dried blood spots, Diagnosis and monitoring of aminoacidopathies, organic acidemias, and fatty acid oxidation disorders. | Allows for pre-symptomatic detection of life-threatening disorders; enables early intervention to prevent disability and death (McHugh et al., 2011). |
| **MALDI-TOF MS** | **Clinical Microbiology** | Rapid identification of bacteria and yeasts from culture, Direct identification from positive blood cultures. | Dramatically shortens time to identification (from days to minutes); facilitates earlier targeted antimicrobial therapy; improves sepsis outcomes (Hu et al., 2019). |
| **MALDI-TOF MS (Emerging)** | **Microbial Typing & Resistance** | Strain typing for outbreak investigation, Direct detection of specific resistance mechanisms (e.g., carbapenemase activity). | Enhances infection control efforts; provides rapid detection of key resistance patterns, informing isolation and therapy (Ota et al., 2019). |

Automation and throughput are also major hindrances. Immunoassay automatic systems dominate large-volume clinical labs, but MS workflows have had high manual sample preparation steps, such as protein precipitation, liquid-liquid extraction, or solid-phase extraction. This not only brings in hands-on time and labor cost but also brings in an element of human error.

Front-end automation solutions are therefore required to integrate, raise throughput, enhance reproducibility, and make MS competitive for high-volume testing (Ho et al., 2020). Finally, analysis of high-complexity data is also more of a problem. The large datasets generated by modern MS, particularly by untargeted high-resolution analysis, need sophisticated bioinformatics tools and skilled data scientists for proper interpretation—a competency not conventionally cultivated within the personnel of a clinical laboratory. Proper processing, handling, and extracting clinically relevant information from such "big data" is a principal bottleneck needing to be overcome to release the complete potential of discovery-proteomics and metabolomics (Drabovich et al., 2019; Aljehani & Alhayek, 2024).

In the years to come, some extremely promising directions are ahead that will go on to transform clinical diagnostics. High-Resolution Mass Spectrometry (HRMS), with platforms including Quadrupole Time-of-Flight (Q-TOF) and Orbitrap instruments, is gradually moving from research into the clinical environment. HRMS permits accurate mass measurement, which enables untargeted "suspension" screening for unknown analytes and retrospective re-interrogation of data many years after the sample has been analyzed, a substantial benefit over targeted MRM strategies. Such a capability is a revolution in areas like clinical toxicology, where there is an ongoing emergence of new psychoactive substances, and for the identification of new disease biomarkers (Kaufmann et al., 2018). Another revolutionary advance is Mass Spectrometry Imaging (MSI), and specifically MALDI-MSI. MSI is employed for imaging the spatial distribution of hundreds of metabolites, lipids, and proteins directly from thin tissue sections.

With spatial context preserved, MSI has huge potential in anatomical pathology for tumor grading, classification, margin evaluation, and unraveling intratumoral heterogeneity in a complementary fashion to traditional histopathology (Fung et al., 2020). In addition, ambient ionization MS techniques are breaking down the walls of complicated sample preparation. Paper spray ionization and rapid evaporative ionization MS (REIMS) is among the techniques allowing one to directly analyze complicated samples, such as tissue or biofluids, with minimal pre-treatment. The iKnife technology, utilizing REIMS in combination with an electrosurgical device, can potentially provide near-real-time tissue identification in cancer surgery and guide the margin of resection and improve patient outcomes (Balog et al., 2013). In the background is the final vision for Point-of-Care (POC) MS. The ongoing development of smaller, more resilient, and easy-to-use mini mass spectrometers holds out the promise of bringing this powerful analytical tool to critical care rooms, emergency departments, or doctors' offices. This revolution could redefine the acute care settings with the provision of ultimate diagnostic answers in minutes, not hours, altering the clinical decision-making dynamics (Snyder et al., 2015).

## Conclusion

The development of mass spectrometry from its initial application in toxicology to its present status as a cornerstone of endocrinology and microbiology is a good example of technological development leading to excellence in clinical diagnostics. LC-MS/MS has supplanted immunoassays as the gold standard for quantitative measurement of hormones and vitamins, with the specificity and accuracy necessary to diagnose complex endocrine diseases and customize therapy. At the same time, MALDI-TOF MS has transformed the laboratory workflow of clinical microbiology, with identification of pathogens at record speed and accuracy, and thus directly improving antimicrobial stewardship and patient outcomes in infectious illnesses. Furthermore, the proven value of MS/MS in newborn screening is still to prevent disaster through early intervention.

The evidence synthesized within this review over the recent decade (2015-2024) unequivocally confirms that MS/MS and MALDI-TOF MS are not just add-on techniques but are at the essence of the work of the modern clinical laboratory. Despite challenges of cost, standardization, and expertise, the path is clear: applications of mass spectrometry will only grow. With ongoing development of the technology towards improved resolution, increased automation, and novel ambient ionization methods, integration into day-to-day clinical practice will become more extensive, further solidifying the technique as an indispensable tool for achieving the aims of precision medicine—delivering the correct diagnosis to the correct patient at the correct time.

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