Review

LC-MS/MS in clinical diagnostics: Opportunities, technical hurdles, and implementation challenges

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Abstract Mass spectrometry (MS) technology, particularly liquid chromatography-tandem mass spectrometry (LC-MS/MS), is emerging as a powerful analytical tool in clinical laboratories. This review explores the current state, challenges, and future prospects of MS integration into routine clinical testing. We discuss the unique advantages of MS, including its flexibility, specificity, and ability to perform multi-analyte measurements, which address several limitations of conventional clinical assays. However, the implementation of MS in clinical settings faces hurdles such as operational complexity, cost considerations, and regulatory challenges.

This paper examines the technical and operational issues that need to be resolved, including sample preparation, method development, and the need for specialized training. We also highlight the roles that various sectors—including education, healthcare, industry, and policy-making—must play in facilitating the widespread adoption of MS in clinical laboratories.

The potential of MS to revolutionize clinical diagnostics is significant, offering improved accuracy, expanded test menus, and new insights into disease processes. The integration of MS into clinical practice represents a crucial step in enhancing patient care and advancing medical science. This review aims to provide a comprehensive overview of the current landscape and future directions of MS in clinical laboratory testing, serving as a resource for laboratory professionals, clinicians, and researchers in this rapidly evolving field.

Key words: LC-MS/MS, clinical laboratory, clinical diagnostics, multi-analyte measurement

1. Clinical Laboratory Testing and Mass Spectrometry Technology

In recent years, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF/MS) has been introduced for microbial identification in clinical laboratories[1)]. Following the 2018 revision of medical fee schedules in Japan, this technology became eligible for insurance coverage. As a result, mass spectrometry (MS), previously recognized primarily as a research tool, is now emerging as a key analytical instrument in clinical testing. In March of the same year, MS was added as a new general term for medical devices under the Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices (PMD Act). Several MS manufacturers have obtained or are in the process of obtaining medical device certification. This trend suggests an acceleration in the adoption of not only MALDI-TOF/MS but also

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other technologies such as Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) in clinical laboratories. LC-MS/MS, in particular, is expected to see increased utilization due to its high sensitivity, specificity, and other performance characteristics well-suited for clinical testing. For this reason, there has been much discussion about the introduction of mass spectrometry technologies such as LC-MS/MS into clinical laboratories[2,3].

During the early stages of the COVID-19 pandemic, many institutions were conducting PCR tests using in-house primers and confirming virus detection through agarose gel electrophoresis. While this approach had a strong research aspect, it was promptly implemented to save lives. In the future, we can anticipate similar scenarios where research-oriented instruments and methods will be effectively utilized to protect lives in various situations. MS, the focus of this article, offers several advantages and can respond to sudden demands. To effectively utilize such technologies when needed, it is essential for us to become proficient in their use on a regular basis.

2. Societal Changes Surrounding Clinical Laboratory Testing

The authors consider MS, particularly LC-MS/MS, to be a potential solution to various challenges facing clinical laboratory testing.

Antibodies, essential for clinical testing, are traditionally obtained from experimental animals. In recent years, there has been growing concern for animal welfare, especially in Western countries. Stricter regulations in many nations have led to increased costs in antibody production[4].

Additionally, the issue of cross-reactivity in antibodies, where they react with similar substances other than the target, often poses problems in clinical testing.

Moreover, recent advancements in deep learning and machine learning techniques have enabled rapid estimation of molecular behavior. This is expected to speed up drug discovery[5]. While this expands the possibilities for patients to overcome diseases, it also implies a rapid increase in the number of analytes that clinical laboratories need to measure.

Given these social trends, there is a need for technologies that allow rapid development of measurement methods and can differentiate between similar substances. LC-MS/MS, which fulfills these requirements, is becoming an essential tool for next-generation clinical testing. In recent years, manufacturers such as Shimadzu[6] and Roche have been marketing specialized LC-MS/MS systems targeting clinical testing. The environment surrounding clinical laboratory testing has been changing dramatically in the past few years, with the infrastructure for utilizing MS gradually taking shape.

Recently, there has been an increasing trend in including correlation data with mass spectrometry measurements, such as LC-MS/MS, in the basic evaluation data of IVD reagents based on immunoassay principles. This trend underscores the high reliability of mass spectrometry data in clinical testing. Furthermore, some manufacturers have begun to use GC-MS/MS for value assignment of calibration reagents, further emphasizing the growing importance of mass spectrometry in ensuring accuracy and reliability in clinical laboratory testing.

3. Differences from Existing Clinical Testing Methods

3.1. Advantages

3.1.1. Flexibility

A key advantage of LC-MS/MS is its flexibility in method development. While chemical reactions or antibody-based measurements often require significant time to establish highly specific assays, MS-based quantification can be relatively easily set up if the properties of the target analyte are known and standard materials are available. This characteristic allows for rapid translation of discoveries in basic medical research to clinical applications.

3.1.2. Specificity

One of the major advantages of MS, especially when combined with separation techniques, is its high specificity. Immunoassays often struggle to accurately differentiate between structurally similar compounds, which can be problematic in clinical settings.

In contrast, LC-MS/MS or GC-MS/MS can separate similar metabolites through column chromatography before mass spectrometric analysis. This capability not only reveals the limitations of existing immunoassays but also demonstrates the ability to measure analytes with high specificity. Furthermore, tandem MS, which connects two MS with a collision cell, can enhance specificity by fragmenting molecules between MSs (Fig. 1). This process can emphasize differences between initially similar molecules, enabling highly accurate differential measurement.

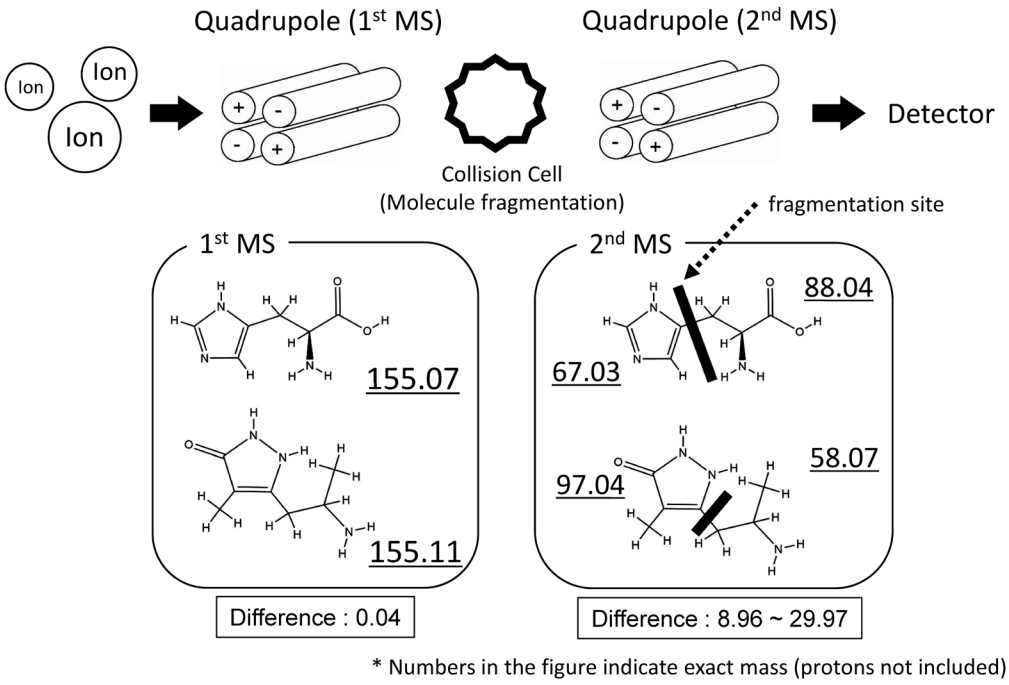

Fig. 1. Advantages of tandem mass spectrometry in determining approximate molecular weight.
An example where two substances of approximate molecular weight that cannot be distinguished by 1stMS can be distinguished by collision cell cleavage of the molecule.

For example, LC-MS/MS can distinguish between sirolimus and everolimus, providing more accurate measurements for these immunosuppressants[7]. Similarly, it can specifically measure 25(OH)D_2 and 25(OH)D_3 without interference from other vitamin D metabolites, offering a more precise assessment of vitamin D status[8].

Recent developments in ion mobility spectrometry capable of separating stereoisomers within the MS promise even more precise analysis of biological components[9], further expanding the potential of mass spectrometry in clinical laboratory testing.

3.1.3. Versatility

Different ionization methods are suited to different types of molecules. Common ionization techniques used with mass spectrometers include MALDI (for MALDI-TOF/MS), Electrospray Ionization (ESI) (for LC-MS/MS), Atmospheric Pressure Chemical Ionization (APCI) (for LC-MS/MS), and Electron Ionization (EI) (for GC-MS). Biological components can be broadly classified according to their suitability for these ionization methods, as shown in Fig. 2. MALDI and ESI are particularly well-suited for ionizing biological components, making them highly versatile methods for measuring such analytes.

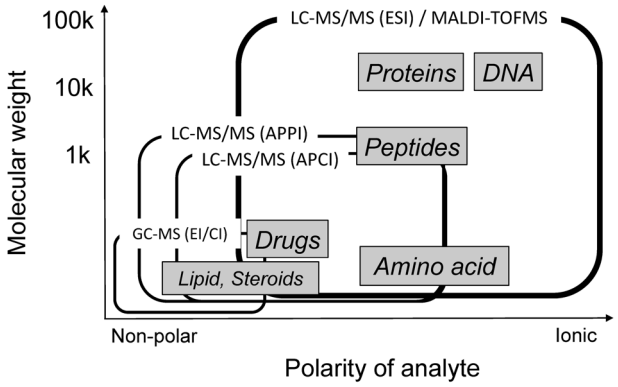

Fig. 2. Analyzable areas of various ionizations.
Characteristics of polarity and molecular weight of biological components and the main measurable areas of various mass spectrometry.

3.1.4. Multi-analyte measurement

A significant difference from conventional clinical testing methods is the ability to analyze multiple analytes in a single run. Traditional clinical tests typically use one specific reagent for each analyte, requiring separate sampling and reagents for each item. In contrast, MS allows for simultaneous quantification of multiple analytes. For example, our group uses GC-MS to measure urinary steroid metabolites[10], simultaneously quantifying multiple items to provide diagnostic information for conditions such as adrenal hyperplasia. Fig. 3 shows the steroids that are increased in

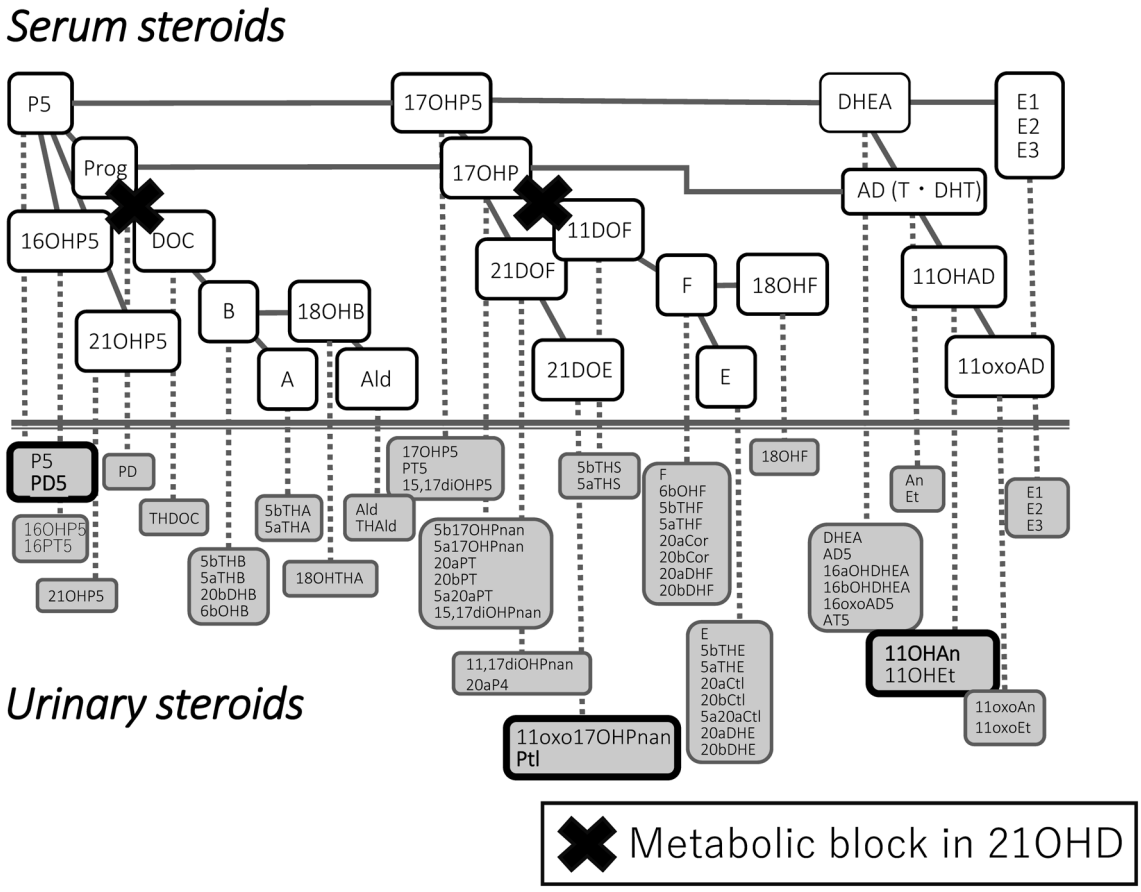

Fig. 3. Overview of blood and urinary steroids and their metabolism in 21-hydroxylase deficiency (21OHD).
The upper part shows blood steroids, while the lower part shows urinary steroids. Each blood steroid is metabolized into the urinary steroid connected by a dotted line. Areas where metabolism is inhibited in 21OHD are marked, and the consequently elevated urinary steroids are shown in bold.

21-hydroxylase deficiency (21OHD). This ability to provide comprehensive profile information is a unique feature of MS.

3.2. Disadvantages

3.2.1. Batch processing

A key advantage of existing clinical testing methods, such as colorimetry, is the ability to measure multiple samples simultaneously. Traditional colorimetric methods typically use a single detector with multiple reaction cells, enabling concurrent processing of samples. In contrast, conventional LC-MS/MS systems can only start analyzing the next sample after completing the current one, resulting in a significantly different throughput compared to automated analyzers using colorimetric methods.

However, recent advancements in MS technology are addressing this limitation. Some innovative approaches now employ multiple columns (LC) connected to a single mass spectrometer[11]. This multi-column LC-MS/MS setup allows for simultaneous processing of multiple samples, potentially increasing throughput.

While this multi-column strategy shows promise for improving MS throughput, it’s important to note that practical implementation may face challenges. These could include cost considerations and potential variations between individual columns. Despite these potential hurdles, this forward-thinking approach represents an exciting development in the field. The future evolution of this technology could significantly impact the efficiency of MS in clinical laboratory settings.

3.2.2. Peak detection

In current clinical testing, liquid chromatography is used for tests such as HbA1c measurement. This test separates hemoglobin using a cation exchange column and calculates the HbA1c value from the peak ratio of the HbA1c fraction. While the instrument automatically calculates peak areas, occasional issues with automatic detection may arise,

requiring human verification. Since LC-MS/MS is considered a highly reliable instrument, its analysis results are treated as highly trustworthy information. Therefore, it is crucial to discuss and explore methods for ensuring data reliability and enhancing the capabilities of medical staff to verify the data.

It is important to note, however, that LC-MS/MS faces similar challenges in peak detection as HPLC, given that the front-end separation in LC-MS/MS is also performed by HPLC. Issues such as peak misidentification and improper peak integration can occur, necessitating careful attention and potentially manual verification, even in LC-MS/MS analysis.

Furthermore, the use of Selected Reaction Monitoring (SRM) in LC-MS/MS introduces an additional complexity. While SRM allows for highly specific detection of target analytes, it also limits the view to only the specified retention times and mass transitions. This focused approach, while beneficial for sensitivity and specificity, can potentially obscure matrix effects that might be more apparent in a full scan chromatogram. The limited visibility of the overall chromatographic profile could make it challenging to identify unexpected interferences or matrix effects that fall outside the monitored transitions.

Given these considerations, it is essential to develop strategies that not only ensure data reliability but also address the potential limitations of SRM in LC-MS/MS analysis. This includes methods to identify and account for possible matrix effects that may not be immediately apparent in SRM mode. By acknowledging and addressing these challenges, we can maintain the highest standards of data quality and interpretation in clinical laboratory settings, while fully leveraging the power and reliability of LC-MS/MS technology.

3.2.3. Insurance reimbursement

In the Japanese healthcare system, insurance reimbursement for clinical tests generally requires the use of in vitro diagnostic (IVD) reagents approved by regulatory authorities. As mass spectrometry-based tests often lack commercially available IVD reagents, they are typically not eligible for insurance reimbursement. This requirement for IVD reagents, rather than specific measurement methods, is the primary barrier to insurance coverage for MS-based tests in Japan.

In contrast, Laboratory Developed Tests (LDTs) are common overseas, allowing labs to develop and profit from their own testing methods. While LDTs have their own challenges, such as lack of standardization and external quality assessment, the approach of developing and implementing advanced measurement methods is considered beneficial for advancing medical care. Recently, discussions about LDTs have begun in Japan, which may lead to changes in the future.

3.2.4. Cost

Cost is a crucial factor in introducing equipment to clinical laboratories. Traditionally, LC-MS/MS has been used as a research instrument. While existing clinical analyzers are also expensive, the business model for selling to clinical laboratories differs significantly from that for research labs. Clinical laboratories perform continuous measurements, so it's common to balance costs including consumables and maintenance contracts. This approach significantly reduces initial costs, making it easier for clinical laboratories to introduce new equipment.

However, simply adopting a business model that recovers costs through consumables and maintenance fees, as is common with immunoassay analyzers, may not be the optimal solution for LC-MS/MS in clinical settings. While this approach could potentially lower initial investment costs, it would likely result in significantly higher consumable and maintenance costs for users compared to current practices. This increase in ongoing expenses could potentially conflict with the previously discussed benefits of laboratory-developed tests (LDTs) in terms of cost-effectiveness.

To address these challenges, a new business model may be necessary. One potential approach could involve manufacturing products using mature, relatively inexpensive technologies, coupled with innovations to extend the lifespan of the equipment. Additionally, instead of relying solely on visible consumables for cost recovery, manufacturers could explore new revenue streams. For example, they could offer value-added services such as add-ons that simplify data analysis or provide SRM datasets for measuring a wider range of substances.

This shift towards a more data-centric, service-oriented model could help balance the need for cost recovery with the unique advantages of LC-MS/MS technology. It would allow clinical laboratories to benefit from the specificity and flexibility of mass spectrometry while managing costs more effectively. Moreover, this approach aligns better with the potential for LDTs and the evolving needs of personal-

ized medicine.

Ultimately, the successful integration of LC-MS/MS into routine clinical testing will require collaboration between instrument manufacturers, clinical laboratories, and regulatory bodies to develop sustainable, cost-effective solutions that maintain analytical quality while supporting innovation in laboratory medicine.

4. Technical Challenges to be Addressed

4.1. Sample preparation

Sample preparation is crucial not only for MS but for all separation analysis techniques. Analyzing samples without proper preparation can reduce column lifespan and lead to contamination of the ion source and ion suppression due to the introduction of unwanted substances. These issues can decrease instrument sensitivity and the robustness of analytical results.

While focusing on sample preparation can improve analytical results, it also increases time, effort, and costs. In clinical testing, where speed and cost-effectiveness are as important as result accuracy, it's necessary to understand the clinically acceptable level of uncertainty and thoroughly discuss and consider the intensity of sample preparation.

In recent years, various manufacturers have introduced automated sample preparation systems[6]. Standalone sample preparation devices often offer a high degree of flexibility and can perform appropriate sample preparation. On the other hand, sample preparation systems integrated with mass spectrometers often feature simplified preparation methods, which may not always provide sufficient sample preparation. However, if it can be confirmed in advance that there are no issues with measurement accuracy, stability, or sensitivity, simplified preparation methods should be welcomed as they can lead to time and cost savings. It is important to note that validation to confirm these aspects must be conducted very carefully.

4.2. Column separation

In clinical applications, column separation requires a balance of high resolution, low pressure, cost-effectiveness, and robustness. High resolution is essential for accurate analyte identification and quantification, while low pressure operations can reduce wear on the system and extend its lifespan. Cost-effectiveness is crucial in clinical settings where high-volume testing is common, and robustness ensures reliable performance over time with minimal maintenance.

Similar to sample preparation, using longer columns, specialized gradients, or unique eluents can lead to better analytical results. However, these approaches can also result in time loss, complexity in method development, and reduced versatility of eluents. It's crucial to carefully consider these factors in column analysis, especially in clinical settings where time and resource management are critical.

For instance, the ability to measure various substances using the same column and eluent is advantageous in clinical laboratories. Higher column robustness can lead to time and cost savings. While UPLC is common in research-oriented LC-MS/MS, lower pressure HPLC systems are less prone to troubles, which can be beneficial in clinical settings where samples of various natures are analyzed. The robustness of the instrument itself is also an important consideration in clinical testing.

It's worth noting that there are ongoing efforts to develop column-free separation techniques, such as ion mobility spectrometry[12]. While these methods are still in development for clinical applications, they hold promise for simplifying the analytical process and potentially reducing maintenance requirements in the future.

4.3. MS-specific pitfalls

As MS detection is based on *m/z* calculated from exact mass and charge number, it presents new pitfalls different from existing measurement methods. Unlike research use, clinical samples may contain unexpected substances due to medication or disease states. While pitfalls in immunoassays using antibodies are widely recognized, MS-specific pitfalls[13,14] are likely to become apparent gradually as more samples are analyzed.

Ion suppression and ion enhancement are often problematic[15]. Care is needed when analysing human samples, which contain a wide variety of substances. In particular, they are susceptible to influence when analysed in a short period of time. In addition, when using deuterium as an internal standard, it is known that exchange reactions between hydrogen and deuterium occur in the ion source[16], so the choice of internal standard is also important. In addition, even when using SRM, which is a feature of MS/MS, specificity may be reduced in some cases. For example, in the phenomenon known as crosstalk, if the product ion size is the same, false peaks may appear[13].

4.4. Maintenance

In clinical testing, instrument downtime can lead to interruptions in medical care, so frequent maintenance may not always be possible. Therefore, greater robustness is required compared to research laboratory use. Additionally, as not all users may be experts in MS, maintenance should be designed to be as quick and easy as possible. Moreover, when used as a medical device, 24/7 support is essential.

5. Operational Challenges to be Addressed

5.1. Choice of analytes

Determining which analytes to measure with this technology is crucial. As shown in Fig. 1, LC-MS/MS can measure a wide range of substances, but it also has its limitations as mentioned earlier. It's necessary to discuss which analytes would best leverage this instrument's capabilities, understanding both its advantages and disadvantages. In particular, the ability to simultaneously measure multiple analytes in a single analysis is a major strength, and it's desirable to choose methods that maximize this feature.

From a clinical laboratory perspective, LC-MS/MS offers a unique advantage in its ability to establish measurement systems relatively easily compared to immunological methods that require antibody production. Many IVD reagent manufacturers only develop analytical methods when they can recover investments such as antibody production. This means that even if a disease marker shows excellent performance, if the patient population is small, it may be difficult to justify the cost of antibody development. LC-MS/MS has the potential to address these hidden demands and solve various social issues by measuring analytes that might otherwise be overlooked due to economic constraints.

When selecting analytes for LC-MS/MS in clinical settings, laboratories should consider not only the technical capabilities of the instrument but also the clinical need, potential impact on patient care, and the ability to fill gaps in current testing methodologies. This approach can lead to the development of novel biomarkers and more comprehensive diagnostic panels that may not be feasible with traditional immunoassays.

5.2. Development of versatile methods

Considering measurement accuracy, sensitivity, and specificity, it would be ideal to optimize columns, eluents, gradients, and sample preparation for each analyte. However, frequently changing columns or eluents can cause problems and lead to downtime. Developing more versatile methods (using the same column or eluent for multiple analyses) can reduce the frequency of these changes, leading to improved convenience.

While the development of completely versatile methods remains challenging, advancements in HPLC technology offer practical solutions. Modern HPLC systems now allow the use of multiple eluents and columns on a single device. These features can be particularly beneficial in clinical laboratory settings where large numbers of samples need to be analyzed. By leveraging these capabilities, laboratories can strike a balance between optimized methods for specific analytes and operational efficiency.

For instance, automated column and solvent switching systems can be programmed to change setups between batches of different tests, minimizing manual intervention and reducing the risk of errors. This approach allows for the use of optimized conditions for each analyte or group of analytes while maintaining high throughput and minimizing downtime. Furthermore, some systems offer parallel analysis capabilities, allowing simultaneous runs with different conditions, which can significantly increase overall efficiency in a clinical laboratory setting.

5.3. Shortage of laboratory staff proficient in MS

As MS is not yet common in clinical laboratories, there is still a shortage of clinical laboratory technicians proficient in MS techniques. To address this issue, the Japanese Society for Biomedical Mass Spectrometry has established a certification system. It is hoped that through such initiatives, correct utilization of the technology will progress.

6. Tasks for Each Sector to Promote Widespread Adoption

6.1. Educational sector

The educational sector needs to provide technical training in MS to future users (medical doctors, clinical laboratory technicians and pharmacist) and raise awareness about the advantages and disadvantages of this technology. While it's essential to have operators who can actually use the equipment, doctors and others who will make decisions based on the results also need to understand the characteristics of this method.

6.2. Medical sector

The medical sector needs to actively incorporate and uti-

lize MS technology. One approach is to use MS as a solution to problems with existing tests. Another is to use MS to rapidly apply discoveries from basic medical science to clinical medicine. Through such activities, it's necessary to unlock the potential of MS technology.

6.3. MS manufacturing and sales sector

Clinical laboratory settings have different needs compared to research settings. Therefore, manufacturers need to develop products tailored to clinical laboratory environments and establish business models that differ from those used for research laboratory sales. As existing MS manufacturers may not be fully familiar with medical settings, cooperation between LC manufacturers already established in clinical testing and MS manufacturers to produce and sell clinical testing-oriented equipment could lower the barrier to entry into the medical field.

6.4. Academic organizations and policy-making/implementation sectors (Government, etc.)

Academic organizations need to promote the use of this new technology while also developing guidelines for its use. These guidelines should be established by experts, cover issues anticipated in clinical laboratory use, and be widely applicable. The Japanese Society for Biomedical Mass Spectrometry has established a “Mass Spectrometry Testing Standardization Working Group” to work towards future clinical laboratory applications.

Furthermore, the policy-making and implementation sectors, including the government, are required to engage in active discussions on how to support and appropriately apply to medical care not only MS technology but also other advanced technologies expected to emerge in the future.

7. Conclusion

MS technology has the potential to complement and address some of the limitations of current clinical laboratory testing methods. While there are several challenges to its widespread implementation, active discussion among various stakeholders is expected to lead to solutions for a range of social issues.

This technology offers unique capabilities that can significantly enhance the field of clinical diagnostics. As we move forward, it's crucial to balance the technical advantages of MS with practical considerations for clinical use. The journey towards integrating MS into routine clinical practice will require collaborative efforts from multiple sectors—education, healthcare, industry, and policy-making.

By addressing the challenges outlined in this review and leveraging the strengths of MS, we can potentially revolutionize certain aspects of clinical testing. This could lead to more accurate diagnoses, better patient care, and new insights into disease processes.

The future of clinical laboratory testing is likely to involve a harmonious integration of traditional methods and advanced technologies like MS. As we continue to develop and refine these techniques, we move closer to a future where personalized, precise, and comprehensive diagnostic tools are readily available in clinical settings.

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