Clinical Chemistry 62:1
134–143 (2016)

Reviews

Applications of MALDI Mass Spectrometry in Clinical Chemistry

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BACKGROUND: MALDI-TOF mass spectrometry (MS) is set to make inroads into clinical chemistry because it offers advantages over other analytical platforms. These advantages include low acquisition and operating costs, ease of use, ruggedness, and high throughput. When coupled with innovative front-end strategies and applied to important clinical problems, it can deliver rapid, sensitive, and cost-effective assays.

CONTENT: This review describes the general principles of MALDI-TOF MS, highlights the unique features of the platform, and discusses some practical methods based upon it. There is substantial potential for MALDI-TOF MS to make further inroads into clinical chemistry because of the selectivity of mass detection and its ability to independently quantify proteoforms.

SUMMARY: MALDI-TOF MS has already transformed the practice of clinical microbiology and this review illustrates how and why it is now set to play an increasingly important role in in vitro diagnostics in particular, and clinical chemistry in general.

MALDI-TOF mass spectrometry (MS)[5] has the potential to make further inroads into clinical chemistry. An extensive literature details MALDI-TOF MS instrumentation, its operation and a broad array of applications. The MALDI process can be used as the front end to other analyzers, but the most common and practical configuration, especially for routine applications, is MALDI combined with a TOF analyzer. In this review we highlight the operational features of MALDI-TOF MS and the benefits these can bring to clinical chemistry. We also illustrate the utility of MALDI-TOF MS by way of practical examples.

The Basics

THE PROCESS

Although the practice of MALDI-TOF MS varies in detail, there are common essential features to its application. In contrast to electrospray ionization (ESI) analysis, which is typically performed through combined LC-MS or LC-MS/MS, any sample preparation for MALDI is decoupled (i.e., performed offline) from the process of sample analysis. The basic components of a MALDI system are illustrated in Fig. 1. Typically a sample (e.g., plasma) is mixed with a matrix solution (see below) and a small volume (approximately 1 μL) is applied to the surface of the MALDI target— usually a stainless steel plate. The solution is allowed to dry on the target and then introduced into the mass spectrometer. Although more complex approaches to matrix/sample cocrystallization have been developed (e.g., dried-droplet, sandwich, and thin-layer methods) that offer advantages for some applications, no one variation is widely adopted and routinely used. With the standard approach, hundreds of separate samples can be applied to a single target provided each occupies a unique location. Sequentially, each of the dried sample spots is irradiated with a pulsed laser beam and the ions generated are accelerated down the TOF analyzer and detected. The ion abundances and their times of flight are recorded, and the signals generated are converted to a typical mass spectrum, i.e., a vertical bar graph in which the position of the peaks on the *x* axis represents the *m/z* of an ion and the height of the bar indicates the relative abundance of that ion. Although ion abundances are typically represented by a peak centroid at a defined *m/z* value, the raw data are distributed across a range of *m/z* values and under ideal conditions approximate a gaussian or Lorentzian distribution. Ion abundances (amounts) can be derived from peak areas or peak heights. As a first approximation, the magnitude of the MALDI-TOF MS signal, like that recorded in most mass spectrometers, is determined primarily by the amount of analyte present.

MALDI-TOF MS analyses can cover an enormous mass range (up to several 100 000 Da) of mass space and

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Received August 11, 2015; accepted November 2, 2015.
Previously published online at DOI: 10.1373/clinchem.2015.239491

[5] Nonstandard abbreviations: MS, mass spectrometry; LC, liquid chromatography; FDA, US Food and Drug Administration; ESI, electrospray ionization; SELDI, surface-enhanced laser desorption/ionization; MSIA, mass spectrometric immunoassay; SISCAPA, stable-isotope standards and capture by antipeptide antibodies; iMALDI, immuno-MALDI; Hb, hemoglobin; BNP, B-type natriuretic peptide.

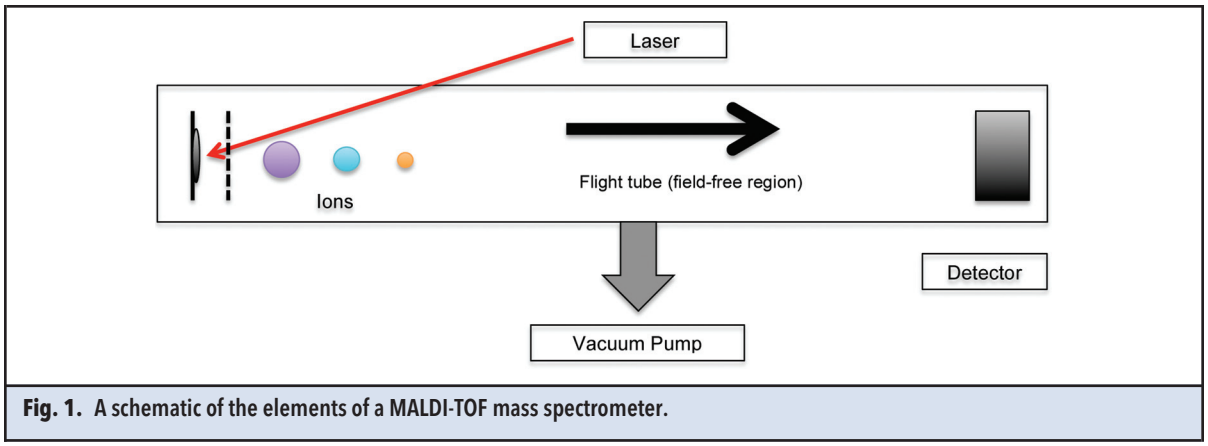

Fig. 1. A schematic of the elements of a MALDI-TOF mass spectrometer.

the spectra comprise primarily singly charged ionic species. These features make MALDI-TOF MS an attractive platform for many applications, including clinical chemistry.

THE SAMPLE

In some cases biological samples can be analyzed directly (i.e., without pretreatment), but more typically 1 or more clean-up/enrichment steps (e.g., filtration, desalting, solid-phase extraction, liquid–liquid extraction, protein precipitation, or immunoaffinity isolation) are performed offline to remove interferents and/or concentrate analytes. The "offline" relationship between sample preparation and analysis facilitates high throughput and makes MALDI practical for the routine clinical analysis of large sample sets such as those processed on the 96- and 384-well microtiter plate scale. Importantly, in contrast to LC-MS–based work flows, the "off line" nature of the process allows preanalytical steps to be optimized without the concerns and constraints imposed by a finite analysis time. Consequently, many samples (hundreds to thousands) can be prepared in parallel, ideally in an automated manner, then loaded into the mass spectrometer and analyzed. The specific sample preparation steps and their order and scale are based on the physical and chemical characteristics of the targeted analyte(s) and the nature of the sample matrix. Sample preparation is also guided by fitness for purpose, i.e., the reproducibility, sensitivity, precision, accuracy, and cost demands of the specific task.

THE MATRIX

An extensive list of matrices has been reported, but in practice, only a few are used routinely. The most common include α-cyano-4-hydroxycinnamic acid, 2,5-dihydroxybenzoic acid, and sinapinic acid. Others have discussed the essential features of the matrix and the mechanism of the ionization process in detail *(1–5)*. Although there is currently no single matrix or matrix preparation protocol that works best for all applications, some chemical companies, instrument vendors, and research laboratories offer useful online guides and specific protocols that are a good starting point for matrix selection and formulation and sample preparation. (These resources and their content change too frequently to direct readers to specific sites, but an online search will quickly identify useful guidelines.) In addition to consideration of the matrix itself, some investigators report the benefits of matrix additives that enhance a specific target signal or suppress background noise *(6–8)*.

Evolution of MALDI and Current Performance Characteristics

MASS RANGE AND MOLECULAR TYPES

Early MALDI studies focused on the analysis of isolated (pure) peptides and proteins, but it was soon evident that complex mixtures could also be analyzed, and with the judicious selection of matrix, matrix formulation, and analyte concentration, almost all molecular types can be analyzed, including lipids (lipidomics) *(9)*, carbohydrates (glycomics) *(10)*, hormones *(11, 12)*, drugs and drug metabolites *(13)*, and nucleotides and nucleosides *(14)*.

In the linear mode, the mass range achievable by MALDI-TOF MS is tens to hundreds of thousands of Daltons. The analysis of low molecular weight molecules (molar masses ≤1000 Da) is possible, but working in this mass range requires some modifications to the standard sample preparation and operating approaches *(15)*.

SPECTRAL FEATURES

Unlike electrospray ionization, the MALDI mass spectrum is not dominated by multiply charged ions for biopolymers. Typically, for any given molecule, the primary ion generated, and therefore its dominant peak in the spectrum, corresponds to the mass of the (singly) protonated molecule: $[M+H]^+$. Multiply

charged ions (e.g., doubly and triply charged forms such as $[M+2H]^{2+}$, $[M+3H]^{3+}$, or more generally, $[M+nH]^{n+}$) are increasingly evident as molecular mass increases, but they are typically low abundance. Infrequently, charge can also arise through adducts formed with cations present in the sample, notably Na^+ and K^+, i.e., $[M+Na]^+$ and $[M+K]^+$. Additional complexity also arises because of the formation of analyte polymers, notably dimers and trimers ($[2M+H]^+$ and $[3M+H]^+$) and formation of matrix adducts ($[M+matrix+H]^+$). These, however, are typically low-abundance ions in the spectrum.

As a first approximation, the magnitude of the MALDI-TOF MS signal is determined primarily by the amount of analyte present, or when multiple components are present in the same sample, their relative amounts determine the relative signal intensities. In practice, however, chemical composition/structure and molecular mass play a role in determining the ionization efficiency and therefore relative peak intensities. Fragmentation is rare except in the case of a few labile protein modifications [e.g., *(16)*]. On modern MALDI TOF MS systems operated in the linear mode, a resolving power of 500–1000 is typical over a wide mass range, but with the inclusion of an ion mirror and with operation in reflector mode, markedly enhanced resolving power is possible (e.g., ≥40 000 for masses below approximately 5000 Da).

REPRODUCIBILITY AND QUANTIFICATION

Discrete (matrix:analyte) crystals comprise the prepared MALDI sample and a survey over the topology is necessary to maximize the analyte-related signal. Because early commercial MALDI-TOF instruments operated at low laser pulse rates (<10 Hz), acquisition times were slow and only a small fraction of the sample surface was interrogated. Consequently, intra- and intersample reproducibility were often poor. Although quantitative studies were reported early in the history of MALDI *(15, 17, 18)*, routine adoption was hampered by instrument limitations. Modern MALDI-TOF instruments incorporate improvements in instrument design and manufacture, including higher repetition rate lasers (1–10 kHz), more sophisticated sample scanning, and improvements in detectors, computers, and data processing. As a consequence, highly reproducible spectra can now be acquired and this makes practical and precise quantification achievable.

SENSITIVITY AND SAMPLE CONSUMPTION

It is difficult to define MALDI "sensitivity" in precise terms given the nature of the process, but numerous groups report detection of low-attomole amounts across a diverse range of applications and masses *(19–21)*. The sample volume applied to the MALDI target is also low (typically 0.5–1.0 μL) and reanalysis of the same target is often possible after storage *(22)*.

SPEED/THROUGHPUT

ESI mass spectrometers are typically coupled directly to a chromatographic system, and with this configuration the separation step is a major contributor to the total analysis time. Separation typically takes several minutes. To offset this, several liquid chromatography (LC) systems are sometimes interfaced to the front end of a single mass spectrometer but this configuration is costly and complex. By contrast, MALDI-TOF MS analysis time is primarily determined by the time it takes to survey the target and gain an acceptable signal-to-noise ratio. By combining a high repetition rate laser with lateral motion of the target to insure continual interrogation of fresh sample, an averaged mass spectrum of good quality (e.g., approximately 10 000 laser shots) can be acquired in a few seconds.

SELECTIVITY

MALDI-TOF MS measures a fundamental physical property of the analyte itself, i.e., its mass or more accurately its *m/z*. Most other widely practiced assays (e.g., ELISA and colorimetric assays) measure signals generated secondary to the analyte, and often as a result of a chemical or physical interaction. Consequently, nonspecific interactions can compromise the selectivity (specificity) of these methods. Multistep analyses—for example MS immunoassays that incorporate 2 orthogonal approaches to target analyte isolation and detection—afford a high degree of selectivity that is unobtainable by other methods. This translates into improved signal-to-noise, increased precision, and ultimately, provided the right standards are available, quantitative accuracy.

IMPLEMENTATION

Concurrent with enhanced performance, modern MALDI-TOF MS systems have become more compact, rugged, and affordable. For example, linear MALDI-TOF systems used for pathogen identification fit easily on a standard laboratory benchtop and are simple in design, reliable, and easy to operate. These instruments are designed for routine use and require no expertise in mass spectrometry. The cost of a linear MALDI-TOF MS system is on the low end of the price range for a mass spectrometer. The switch to MALDI-TOF MS identification in one clinical microbiology laboratory was estimated to reduce annual operating costs by half *(23)*. At least 2 systems are now US Food and Drug Administration (FDA) approved, and as a consequence, we predict the increasingly widespread adoption of MALDI in clinical microbiology. This will lead to economies of scale and the cost of low-end systems is likely to fall. Operating and maintenance costs are modest relative to those for other

mass spectrometers, or for that matter, compared with most analytical methods. The cost per analysis is also low.

Current Applications of MALDI

There have been some unfortunate missteps for MALDI and its adopters in its 27-year history *(24)*. Most notably, a variant of MALDI introduced by Hutchens and Yip *(25)* and referred to as surface-enhanced laser desorption/ionization (SELDI), was commercialized and widely promoted as a tool for biomarker discovery. One high-profile report based on this approach *(26)* was subsequently invalidated *(27, 28)*, but thankfully, the field has (slowly) recovered from this misstep. There are now numerous near-clinical and clinical applications of MALDI-TOF MS, some of which we highlight in the sections that follow. We focus on several emerging areas of activity and exclude discussion of the more mature applications of MALDI-TOF MS. For example, the identification of bacterial and fungal isolates from a single colony is unquestionably the single most important clinical application of MALDI-TOF MS at this time. This strategy, based on the mass assignment of characteristic bacterial/fungal peptide and protein profiles, has been extensively reviewed by others *(29–33)*. MALDI-TOF MS is also routinely used to identify nucleic acid sequence changes (e.g., mutations, single-nucleotide polymorphisms, insertion/deletion, alternative splicing), quantitative changes (e.g., copy number variation, gene expression, allele expression), and for identifying modifications (e.g., methylation of genomic DNA, posttranscriptional modification of tRNAs and rRNAs). These measurements, now commercialized by at least one vendor, have been detailed in numerous reviews *(14, 34, 35)*. Similarly, the application of MALDI-TOF MS to define the distribution of specific compounds on the surface of a tissue sample (imaging) is a very active area of research that has been reviewed by others *(36, 37)*. We also exclude discussion of discovery studies and basic research efforts because the methods that underpin these are frequently not applicable to high-throughput, cost-effective analysis and, in most instances, the molecular targets are yet to be validated.

Representative Near-Clinical and Clinical Applications

Here we focus on the potential of MALDI TOF MS in 2 areas: targeted analyte detection/quantification and molecular fingerprinting.

TARGETED ANALYTE DETECTION AND QUANTIFICATION

In some circumstances the detection/quantification of abundant analyte(s) can be performed in a complex biological matrix with minimal or no extraction [e.g., *(38)*]. There is, however, a substantial sensitivity enhancement following removal of other sample components that lead to concentration-dependent adduct formation, impaired crystallization, and/or ion suppression (competition among species for sufficient interaction with the matrix to promote ionization). Extraction is therefore required for the detection and/or quantification of many analytes. We discuss these 2 approaches separately below.

DIRECT ANALYSIS

Direct analysis is fast, reduces labor/costs, and eliminates steps that might otherwise add variance or bias to the procedure. In practice, however, analyses of complex biological samples without any pretreatment typically yield a signal only for the most abundant components. There are, however, numerous examples of direct MALDI analyses of biological samples, including untreated urine, serum, blood, tear, saliva, and hair. Some of these applications are quantitative (or semiquantitative) in nature, whereas others are qualitative. Several examples are illustrated below.

Protein glycation. Determination of the extent of protein glycation, most notably hemoglobin glycation, is frequently used in the diagnosis and monitoring of diabetes mellitus. Glycohemoglobin can be accurately determined by MALDI-TOF MS analysis of a diluted whole blood sample. Results correlate with hemoglobin (Hb) A_{1c} values determined by alternative validated methods. Traldi and colleagues *(39)* first demonstrated the potential of this approach, but modest mass resolution made it difficult to accurately measure the relevant peak areas. Consequently, precision was compromised, and the authors concluded that "in our opinion, at this stage MALDI/MS cannot be proposed as a routine tool for HbA_{1c} measurements, mainly because the sample preparation phase is not automated" *(40)*. By adoption of leading-edge linear MALDI-TOF MS systems, this application has recently been readdressed *(41)*. In this study, glycohemoglobin was calculated from both the glycated α- and β-subunit by comparing the signal from the unmodified species to that of the unmodified species +162 Da (glucose). Positive ion spectra of whole blood (diluted 1:2000) were generated using sinapinic acid matrix and calibrated against the M^{1+} and M^{2+} ions of hemoglobin α and β subunits. Representative data are shown in Fig. 2. Results were calculated as the ratio of the percent total glycation of each chain using the following formulas:

$$100\ [I_{(\beta+162)}/(I_\beta + I_{(\beta+162)})] \text{ and}$$

$$100\ [I_{(\alpha+162)}/(I_\alpha + I_{(\alpha+162)})].$$

Results were linear over the range 1.4%–18% glycation with CVs <1.7%. MALDI-TOF MS detects all glycation of the α- and β-subunit of hemoglobin, but a

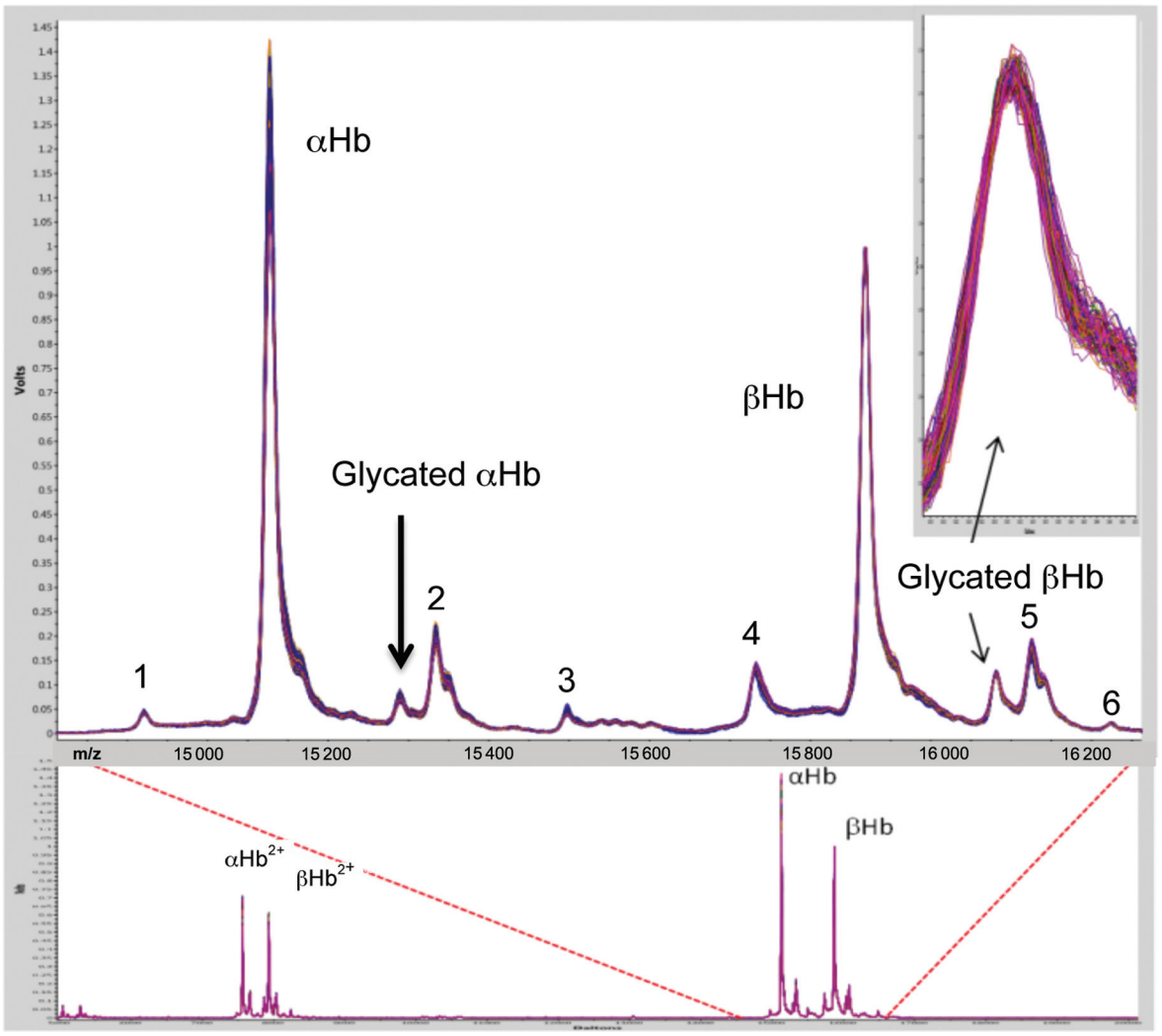

Fig. 2. Overlay of 80 replicate analyses of a single whole blood sample diluted to a ratio of 1:2000.

The spectra show the dominating Hb^{1+} and Hb^{2+} species (used as calibrants). Other peaks can be assigned to hemoglobin marked 1 through 6 as follows: 1, αHb missing C terminal arginine; 2, sinapinic acid matrix adducts to αHb; 3, m/z^{2+} species of the αHb–βHb dimer; 4, βHb missing C-terminal histidine; 5, sinapinic acid matrix adducts to βHb; 6, glutathione modification to βHb.

direct relationship was established between the MALDI-TOF MS method for the determination and the LC method for determination of Hb A_{1c}. Additionally, the strong correlation between glycation of the α- and β-subunits provided a QC check for the determination.

Newborn screening. MALDI-TOF MS is also making inroads into the analysis of dried blood spots for newborn screening. A large cohort (n = 844) of dried blood spots collected from newborns was analyzed by MALDI-TOF MS as a first-tier screen for variant Hbs, diagnostic sickle cell disease, and thalassemia *(42)*. The dried blood spots were analyzed directly at 1:50 dilution in MALDI matrix. The objective was to monitor the mass and relative abundances of the primary Hb α and β subunits, and of the hemoglobin S subunit—indicative of sickle cell disorder. The Hb S subunit results from a single amino acid mutation of G to V and is marked by a decrease in mass of 30 Da. The authors concluded that this approach resulted in a substantial increase in throughput at a marked reduction in cost per unit analysis.

Hair analysis. MALDI-TOF MS has also been used for the direct analysis of human hair. Initial applications have focused on using MALDI-TOF MS to detect drugs such as methoxyphenamine *(43)*, cocaine *(44)*, and ket-

amine *(45)*. The analysis does require that the hair sample be cryogenically sliced to expose the pulp center, and thereafter chronological information is available. Once sliced, the hair is placed on the MALDI target and matrix is applied. More elaborate preparation procedures aimed at enhancing the extraction of specific target compounds have recently been reported *(46, 47)*. The incorporation of compounds into the hair root has been studied over the course of weeks *(48)*. Although most analyses are performed on single strands of longitudinally sliced hair simply plucked from the head, a recent investigation used daily shaved beard hair *(49)*. Conventionally hair analysis for drugs and metabolites uses GC-MS or LC-MS/MS, but these methods are time-consuming and typically require multiple runs to produce the same information that can be obtained from a single MALDI analysis. Drug testing in hair is increasingly used in court, and this has prompted the development of validated MALDI-TOF MS assays for the detection of controlled substances in hair *(50, 51)*.

Proteinuria. MALDI-TOF MS analysis of human urine has been proposed as an approach to the rapid screening of proteinuria *(52)*. The authors reported a limit of detection for albumin analysis of 10^{-6} mol/L, and they suggested that the relative intensities of singly and doubly charged albumin ions varied with albumin concentration. The authors were therefore able to prepare a calibration curve based exclusively on the ratios of these 2 ions and use this to calculate urinary albumin concentrations in patient samples. The authors suggested that, although urinary dipstick testing is currently the most widely used diagnostic method for albuminuria, a MALDI-based approach is less susceptible to interferences, easier to automate, and provided more accurate results *(53)*. More recently the same group has used trypsin digestion combined with MALDI-TOF MS analysis for the rapid diagnosis of albuminuria *(54)*.

Oligosaccharidoses. MALDI-TOF MS analysis of urine has been used in the diagnosis of oligosacharidoses, a group of inherited metabolic disorders that cause the excretion of incompletely degraded oligosaccharides in urine *(55)*. In this application urine samples were analyzed without pretreatment and the investigators used characteristic MS and MS/MS molecular profiles to identify fucosidosis, aspartylglucosaminuria, GM1 gangliosidosis, Sandhoff disease, α-mannosidosis, sialidosis, and mucolipidoses type II and III. The approach is rapid, performed in a single step, and can be automated for routine urinary screening of oligosacharidoses.

ANALYSIS FOLLOWING EXTRACTION/ISOLATION

Liquid and solid phase extraction and all manner of chromatography and electrophoretic methods have been applied to sample cleanup before target analyte detection and/or quantification. With these strategies quantification proceeds in a conventional manner, and involves incorporation an internal standard early in the process. The internal standard should be a good molecular mimic of the target analyte so that any factor that affects the analyte signal will also affect the signal of the internal standard to the same degree. As a consequence, the ratio of the 2 signals will exhibit less variability than the signal derived from the analyte alone. Ion heights/areas are measured and converted to amounts by reference to a calibration curve prepared at the same time and under identical conditions.

The most powerful and commonly adopted iterations of this general approach are discussed below. Each is a multistage process that uses immobilized antibodies to capture and concentrate target analytes (either proteins or peptides derived from them) from biological fluids before selective detection by MALDI MS. In this way each is analogous to ELISA except the second antibody is replaced by highly selective MS detection. This second step is therefore orthogonal to the first step of selective antibody binding, making the assay highly specific (i.e., 2 orthogonal selectivities are combined together). We anticipate growing interest in approaches of this nature.

Mass spectrometric immunoassay (MSIA). In MSIA, proteins are kept intact and the antibodies are immobilized in a pipette tip. Antibodies immobilized in pipette tips make it possible to process very large sample volumes (and numbers) in a parallel format (e.g., 96-well array) and they facilitate the process of assay development: i.e., optimization of the conditions for antibody/antigen interaction, binding kinetics, sample volumes, and incubation times. Optimized MSIA work flows are robust, high throughput, and can form the basis of cost-effective and precise quantification at picogram amounts of analyte per milliliter of sample. For example, a recent study on the analysis of insulin-like growth factor-1 by MSIA reports the processing of more than 1000 samples in a single day *(56)*. The limit of detection and limit of quantification were 1.5 μg/L and 5 μg/L, respectively, with intra- and interassay CVs of $<$10%. This same publication includes a detailed analysis of cost per analysis by MSIA and convincingly demonstrates that this is on par, or better, than that of current FDA-approved ELISA approaches.

A major benefit of MSIA is that proteoforms of the target analyte are retrieved by the immobilized antibody and during the MALDI-TOF MS detection/quantification step. These are detected as separate entities and can be quantified individually. The primary form of the protein is typically measured in absolute terms because its

amount is measured relative to the reference standard incorporated in the assay. Amounts of any proteoform(s) are determined in relative terms (i.e., a ratio of peak areas because standards for these are typically not available to include in the assay).

Although some dispute the clinical significance of proteoforms, Niederkofler and colleagues provided compelling evidence to demonstrate their importance. These authors developed an MSIA for the quantification of B-type natriuretic peptide (BNP) at single-digit picogram per milliliter concentrations *(57)*. When this assay was applied to patients with heart failure, 5 molecular variant forms of BNP were identified and concentrations of BNP 1–32 (active form) were found to be very low. Contrastingly, when BNP concentrations were measured with a commercial point-of-care enzymatic-based assay (Biosite), all patients showed high BNP concentrations. The investigators concluded that the majority of BNP present in the blood of patients with heart failure was present as an inactive propeptide. The investigators used these findings to explain the "natriuresis paradox," wherein patients that have increased concentrations of ELISA-measured BNP benefited from infusion with bioactive BNP 1–32. Subsequent independent studies show that multiple pathways are involved in the dysregulation of proBNP in heart failure, and that both the processing of proBNP and the downstream degradation to BNP 1–32 are clinically significant *(58)*.

Nelson and colleagues have made extensive use of MSIA to quantify a range of clinically relevant peptides and proteins. In studies of RANTES, for example, Nelson and colleagues detected more than 19 variants, including the dipeptidyl peptidase IV truncated variant and other modified forms showing N- and/or C-terminal truncations, oxidation, glycation and glycosylation [Oran et al. *(59)*]. It is important to acknowledge, however, that the clinical significance of these proteoforms is not yet established. Nevertheless, the scale of some of these studies is impressive and so too is the heterogeneity evident at the population level. This has led to the concept of "population proteomics" *(60–62)*. We anticipate growing interest in the biological relevance of proteoforms and the application of this highly selective analytical approach and variants of it. The principles and practice of MSIA have been reviewed *(63)* (see Fig. 3) and the MISA tip technology has been commercialized.

Stable-isotope standards and capture by antipeptide antibodies (SISCAPA) MALDI TOF MS. A variant of MSIA, referred to as SISCAPA *(64)*, involves cleavage of the intact polypeptide to peptide fragments before antibody cleanup and detection/quantification by MALDI MS. This LC-free approach is growing in importance because it is reportedly accurate, robust, and high throughput, but on the downside, because antipeptide antibodies are used, nonspecific binding to other serum peptides may occur, which can lead to either interferences or ion suppression of the MALDI signal. Further, because of the trypsin cleavage step, there is some loss of information content with this strategy *(65)*. Variations of this general approach utilizing electrospray MS are the most common manifestation of SISCAPA to date.

Immuno-MALDI (iMALDI). Analogous to SISCAPA, iMALDI involves the affinity capture of target peptides derived from an enzymatic digest of a sample, followed by the analysis of the affinity beads directly on the MALDI target *(19)*. For the determination of peptide concentrations, stable-isotope–labeled standard peptides can be incorporated in the process. Peptide concentrations can then be converted to protein amounts. Specificity is determined by the antibody, the ions monitored, and can be enhanced further by performing MALDI-MS/MS.

Fingerprinting (Profiling) Intact Complex Biological Samples

With fingerprinting, the objective is to retain the complexity of the biological sample. Most MALDI profiling studies have targeted proteins and peptides in biofluids (e.g., plasma or urine) after sample processing, whereas others have studied biological tissues and used a process of MALDI imaging MS for spatial analysis of protein and peptide signals.

As commonly practiced, profiling does not attempt to identify or quantify specific components of the sample; rather, it is used to identify features (*m/z* values and their relative peak heights/areas) within spectra that distinguish between distinct groups (e.g., spectra derived from control vs disease populations). Although many have explored this approach over the last 2 decades, and over 150 MALDI-profiling reports have been published, few tests of clinical utility, or even clinical potential, have emerged. Profiling tests have, however, been commercialized [e.g., *(67)*] and there is the potential for other studies to ultimately deliver tests of clinical value [e.g., *(68)*]. Nevertheless, it is important to acknowledge that large-scale clinical validation studies are a critical step before any pattern recognition approach can be routinely adopted in human sample.

In large part, the failure of MALDI profiling efforts targeting biological fluids (e.g., plasma) arises because only a handful of proteins make up most of the total protein mass of these fluids. Consequently, features derived from these components dominate the spectrum and some of the more interesting proteins are masked. Pretreatment steps aimed at removing these abundant proteins introduce variance and confound comparisons. In addition, deficiencies with clini-

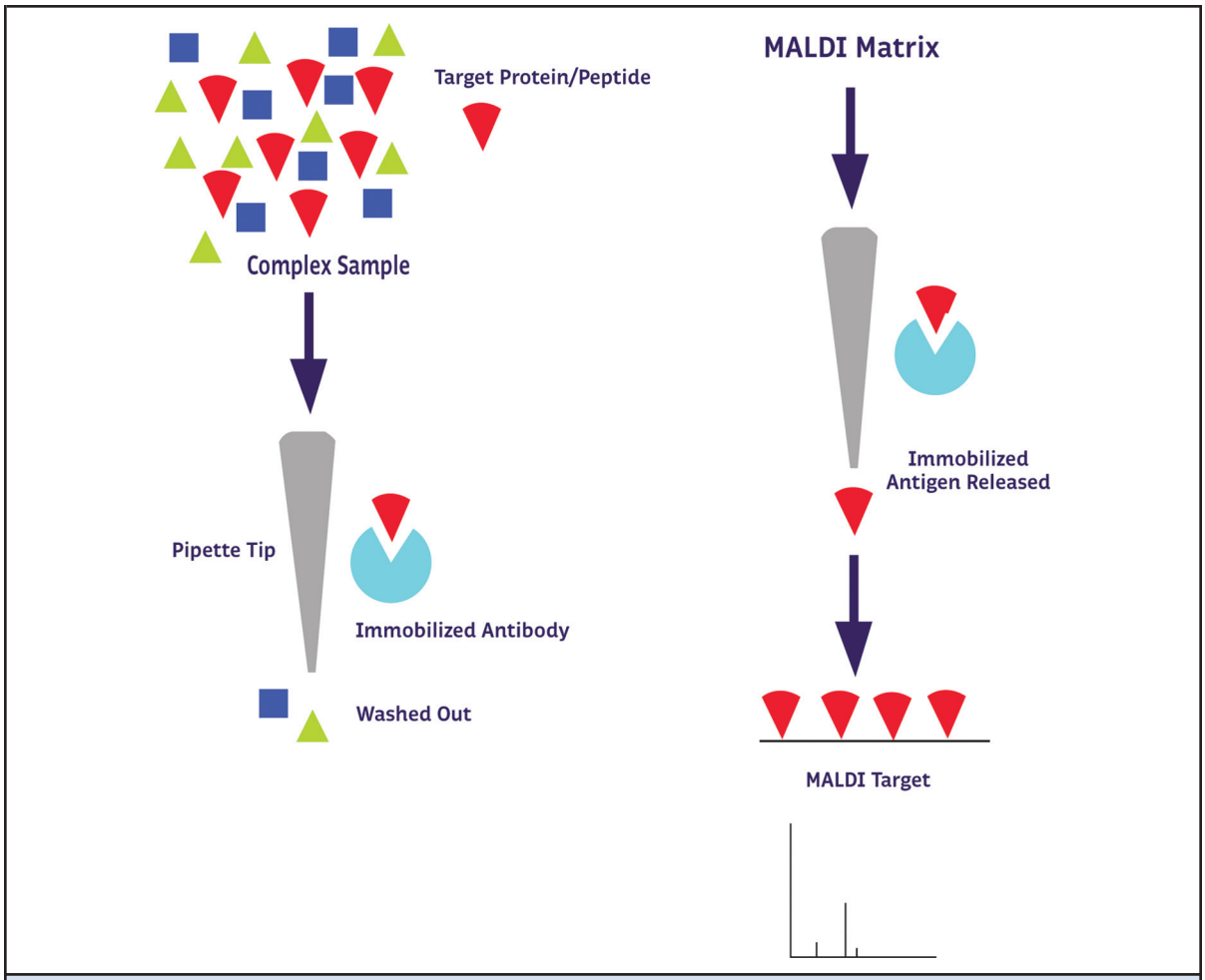

Fig. 3. An illustration of the steps involved in the target mass spectrometric assays discussed in this review.
Note that MSIA and SISCAPA are essentially the same, except that MSIA is based on the analysis of intact proteins whereas SISCAPA digests protein to peptides as a first step in the process. The elements of this Fig. apply equally well to both strategies.

cal samples and study design (e.g., underpowered) have compromised some, if not many investigations.

The question, however, is not whether MALDI profiling is of value, but how can we best bring this basic strategy to bear on important problems and derive clinical value? It is a simple modification of profiling that is used in the rapid and accurate identification of microorganisms. The primary difference is that, instead of making comparisons between groups of spectra, an algorithm is used to match a sample spectrum against a curated database of mass spectra *(29–33)*. In another iteration of the same basic strategy, MALDI profiling of tissue samples allows sensitive and selective spatial analysis and has the potential to provide data-driven (unbiased) histological analyses founded on the presence, amount, and distribution of biomolecules within a tissue *(66)*. Although there is no question that distinguishing biochemical subtle differences between closely related clinical groups is a demanding challenge, we anticipate that further variants of this strategy will likely deliver additional powerful clinical tools.

Conclusions

MALDI-TOF mass spectrometers have evolved substantially since the technique was first introduced over 25 years ago. Current systems are affordable, operate over an extended linear dynamic range, and are simple in design, easy to operate, and rugged. MALDI-TOF MS is therefore an ideal platform for sensitive, high-throughput, and cost-effective assays, not only for intact proteins, but for a wide range of other analytes. MALDI-TOF MS has transformed the practices of clinical microbiology and genotyping single-nucleotide polymorphisms, and additional applications are imminent. Multivariate MALDI-

TOF MS analyses come at no extra cost in terms of analysis time and sensitivity, and we predict more tests will take advantage of these capabilities in the future, whether for conventional multicomponent quantification or biomolecular pattern matching.

There is also increasing recognition of the role protein modifications play in aberrant biology and disease processes. The inability of peptide-centric proteomic methods to identify and quantify these may not be significant when building an understanding of function, but it may be folly when the objective is to understand dysfunction. Analytical methods that can detect and quantify these subtle modifications (proteoforms) may be increasingly critical for the development of sensitive and specific diagnostics in the future. Intact protein analysis by MALDI-TOF MS offers this major advantage, and for this reason alone, we anticipate seeing increasingly use of MALDI-TOF MS in clinical testing.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: M.W. Duncan, Biodesix Inc.
Consultant or Advisory Role: M.W. Duncan, SimulTOF Systems and Biodesix Inc.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: None declared.
Expert Testimony: None declared.
Patents: M.W. Duncan, a patent application has been submitted to cover aspects of work discussed herein.

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