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EXPERT OPINION

Can LC and LC-MS ever replace immunoassays?

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Immunoassays have been the technology of choice for the analysis of biomolecules for many decades across a wide range of applications in research, diagnostics and infectious disease monitoring. There are good reasons for the wide adoption of immunoassays but even such a well established and characterised technique has limitations and as such investigators are looking at alternative technologies. One such alternative is liquid chromatography (LC) and, more specifically, liquid chromatography coupled with mass spectrometry (LC-MS). This article will review both immunoassay and LC and LC-MS technologies and methodologies and discuss the advantages and limitations of both approaches. In addition, the next developments that will need to occur before there is widespread adoption of LC and LC-MS technology preferentially over immunoassays will be examined.

Keywords: immunoassay, liquid chromatography-mass spectrometry, ELISA, LC-MS, diagnostics.

Introduction

This review is not intended as a summary of research and development of immunoassays and liquid chromatography-mass spectrometry but rather as a brief summary of the pros and cons of each technology applied to the quantitative measurement of biomolecules primarily in biofluids.

The ability to detect and quantitate biomolecules revolutionised science in the twentieth century and continues to this day. It allows scientists, for example, to conduct research to find and validate biomarkers, but has found its most prominent role in diagnostic applications where it is used to monitor the presence and levels of biomolecules in human samples for the diagnosis and monitoring of disease, in food and beverages to ensure food safety and authenticity and in the environment to monitor the presence and levels of contaminants of ground, waste and drinking water. The growth in personalised or precision medicine will be accompanied by an increased need to detect and quantify panels of biomolecules as biomarkers, as well as a range of drugs taken as treatment or as a measure to delay or prevent onset of disease, following the paradigm of the right drug for the right patient at the right time and importantly at the right dose.

Increased health and safety regulations demand more testing of food and beverage and environmental samples. With increasing need for biomolecule detection and quantitation comes the demand for high-throughput, lower cost per sample analysis and more accurate results. Traditionally, immunoassays have been the technology of choice for the detection and quantitation of biomolecules. However, over the past two decades alternative technologies have been shown to offer a complementary role to immunoassays. Liquid chromatography-mass spectrometry (LC-MS) is one such technology and for some applications has been shown to offer a powerful alternative to immunoassays. With the increasing demands for routine biomolecule quantitation coming from a broad range of fields, are immunoassays the best solution to keep pace with the increased throughput needed, demand for lower cost per sample and improved accuracy

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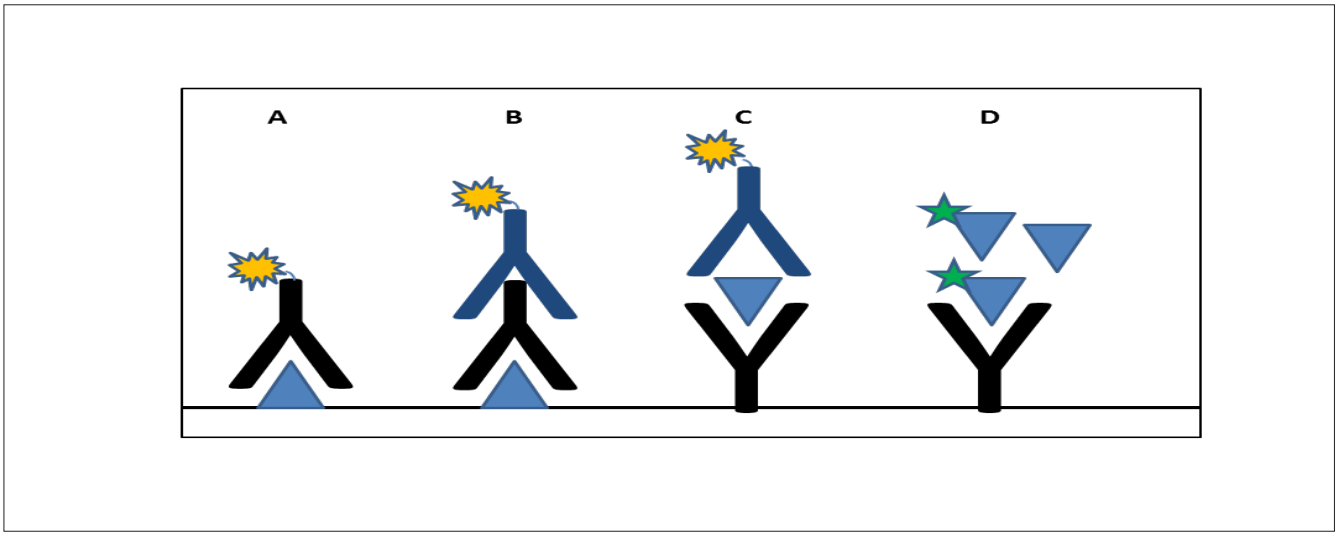

Figure 1. ELISA Formats. A) Direct ELISA, B) Indirect ELISA, C) Sandwich ELISA, D) Competitive ELISA

requirements or is LC and LC-MS the solution to meet these needs longer term?

Immunoassays

The immunoassay was first described by Yalow and Berson [1] in 1959. This was a radioimmunoassay (RIA) using radiolabelled insulin to determine the concentration of insulin in human plasma. It was not until 1971 that Engvail and Perlman [2] introduced the ELISA (enzyme-linked immunosorbent assay) which most people are familiar with and is commonly used today. In this ELISA approach, the antigens were immobilised on to a microplate well, incubated with antiserum and then presence and concentration of antibody determined using an enzyme-linked anti-immunoglobulin antibody. ELISA is a common immunoassay platform and has been developed to offer four main variations which are outlined here.

In the direct ELISA, the sample containing antigen is incubated and adsorbed to the surface of a microplate well. An antibody with bound substrate is then added to bind to the antigen. An indirect ELISA is similar in that the antigen is adsorbed to the microplate well and the antibody applied, however this antibody has no bound substrate. Instead a secondary antibody from a different species is applied with a conjugated substrate. The sandwich ELISA is the most utilised of ELISA assays as it offers higher sensitivity than the direct and indirect approaches. In the sandwich ELISA approach, a capture antibody specific to the antigen of interest is adsorbed to the well. The antigen containing sample is then added and binds to the antibody. A second, substrate conjugated, antibody specific to a different epitope on the antigen is then applied. The final ELISA format is the competitive ELISA where two antigens compete for a limited number of antibody binding sites. One of these antigens will be the antigen of interest; the other is usually the same antigen with biotin attached and so competes for the same antibody. The signal generated will be inversely proportional to the amount of antigen.

The ELISA format, typically the sandwich ELISA, is the basis for most immunoassays and over the years modifications and extensions to this format have been made to improve performance of the immunoassay. The major focus for immunoassay improvement has been on sensitivity. Colorimetric based reporters have been replaced by fluorescence to increase signal and other methods have been developed to amplify the signal including immuno-PCR [3] and electrochemiluminescence (ECL) [4]. There have also been developments in throughput with multiplexing [5]. Here multiple analytes are measured in the same sample by using a variety of specific antibodies bound to a surface that is applicable to automation, such as a magnetic sphere or a protein microarray. Another advance has been to move away from the core concept of the immunoassay, the antibody, and use other affinity ligands such as aptamers [6]. Irrespective of these developments, the underlying immunoassay concept remains little changed, in that an affinity ligand, in most cases an antibody, is used for detection and quantification of the molecule, in a sandwich ELISA based approach.

Advantages of Immunoassays

With a technique that has been utilised for over 50 years, there are obvious advantages to using immunoassays:

- Ease-of-use – Immunoassays are relatively straight-

forward to perform and to interpret the data. There are therefore low training requirements for operators. In many cases, the immunoassay is also available in kit format from commercial vendors reducing complexity further.

- Acceptance – In regulated environments especially, there is the need for the assay to be fully validated and approved as fit-for-purpose. For example in use in a particular diagnostic application. Immunoassays are approved for use across a broad range of applications and both new and existing laboratories will tend to conform to the status quo. In addition, with such an accepted and practised technique, the protocol and any likely potential problems will have been well characterised to aid in troubleshooting when performance comes under par.
- Equipment costs – A basic immunoassay can be performed without the use of expensive capital equipment, the only necessity being a microplate reader. Liquid handling robots to improve automation add additional costs as do multiplexed immunoassay instruments.
- Throughput – Due to the microplate format of most immunoassays, it is possible to process many samples in parallel to give relatively high throughput. Multiplexed immunoassays offer the ability to also assess multiple analytes in each sample.
- Sensitivity – Immunoassays, coupled with the advances in signal amplification, typically offer a high level of sensitivity. Due to the immuno-selection of the analyte of interest other non-binding analytes are removed and any masking of low-abundance proteins by highly abundant proteins is limited aiding sensitivity.

Immunoassay Limitations

The development of new technologies for biomolecule detection and quantitation, has highlighted some of the limitations of immunoassays:

- Selectivity – Using antibodies and affinity ligands stipulates that selectivity of the immunoassay will only be as good as that offered by the antibody. Generating highly selective antibodies is difficult and any small changes in biomolecule configuration can diminish the ligands binding efficiency. Selectivity can also be affected by autoantibodies [7] and human anti-reagent antibodies [8], which can lead to false results with serious outcomes [9], and matrix effects since immunoassays are generally not coupled with sample extraction or separation. With multiplexed immunoassays, cross-reactivity between antibodies has to be investigated and eliminated to prevent false readings. Finally, the process of producing a selective and specific antibody is time consuming and on average takes 2-3 months.
- Analyte detection – To be able to detect the analyte requires the availability of a selective antibody; two of them for a sandwich ELISA. Antibodies can be generated to protein-based antigens and biomolecules, but is very difficult, and in some cases impossible, for other non-biomolecules which can limit their use in some areas. Potentially more limiting though is the fact that many antibodies cannot distinguish between small differences in antigens such as different protein isoforms or altered post translation modification (PTM) status [10]. These small differences in the analyte can often have profound biological consequences or diagnostic implications and subsequently need to be detected and adequately quantitated.
- Sample volumes – An immunoassay such as ELISA typically requires 100-200 µl of sample which is a problem with scarce samples such as ocular fluid for example. Multiplexed immunoassays and other developments use lower sample volumes, but they are still high relative to competing technologies.
- Cost-per-sample – Although the equipment costs are low with immunoassays, the reagent costs do mean that the overall cost per sample and daily running costs may be quite high. Typically the cost per sample is dependent on a number of factors such as the platform used, the level of multiplexing and antibody royalties, however an average cost per sample for a single analyte ELISA is in the range of $4-5, with the majority of the cost, up to 80%, coming from the antibody.
- Reproducibility – The immunoassay is a multi-step process, without automation, a very manual process and centred around an unstable, dynamic and relatively complex biological molecule – the antibody. Intra and inter assay and lab variability is relatively high and variability can also exist across immunoassay platforms. Intra and inter lab variability is dependent on the immunoassay platform used, but the typical range of deviation is 5-15% for intra assay and up to 15-30% for inter assay variability. An example that evaluated immunoassay inter assay variability showed 4-49% for estradiol-17β and 6-45% for progesterone [11].
- Multiplexing – While this has become possible with immunoassays, obvious practical limitations exist as to the degree of multiplexing possible due to antibody cross-reactivity and the time required investi-

gating the potential for cross-reactivity.
- Assay time – While having the ability to run multiple samples in parallel and multiplexing increases throughput, the actual time to run this assay from start to finish can take 2-3 hours with the antibody-antigen binding and wash steps.

Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS as the name suggests is a combination of two techniques; liquid chromatography and mass spectrometry. Liquid chromatography functions to separate out analytes in complex mixtures, in essence simplifying a mixture. In liquid chromatography, the sample is applied to a column (or stationary phase) by the mobile phase. The analytes in the sample will have different affinities for the stationary phase and will bind weakly or strongly or not at all depending on that affinity. Changing the mobile phase over time, by for example increasing the relative proportion of one of the mobile phase components, to reduce affinity to the stationary phase then causes the analytes to elute with the weakest bound eluting first and the strongest last to give separation of the analytes. The analytes can then be detected with a range of different detectors, one of which is a mass spectrometer. In mass spectrometry, analytes and chemical species in the sample are ionised and the ions separated based on their mass-to-charge ratio and subsequently detected. The results are displayed as a mass spectrum with the relative abundance of detected ions as a function of the mass-to-charge ratio. The analytes in the sample can then be identified by correlating known masses to the measured masses with, in addition, on some mass spectrometers, such as triple stage quadrupoles (QqQ), quadrupole time-of-flight (QTOF) and the orbitrap mass spectrometers, the capability to perform tandem MS which breaks apart the molecule of interest (the precursor) to produce diagnostic ions (the products) which can form further evidence of correct identification and in addition contribute to accurate quantification.

Coupling together liquid chromatography and mass spectrometry allows for the separation of complex samples to greatly reduced complexity at the time of mass spectral analysis with analytes being passed sequentially in to the mass spectrometer for identification and quantitation and hence an alternative to immunoassays. LC-MS assays can investigate a range of biomolecules and small and large molecule drugs. LC-MS can directly measure intact proteins, however in complex samples there are insufficient differences in the proteins' physiochemical properties to give good separation in liquid chromatography. Hence, in most LC-MS experiments, the proteins of interest are enzymatically digested, typically with trypsin, to produce smaller peptides to aid chromatographic separation prior to mass spectrometry and additionally the mass spectrometer is more sensitive to peptides than proteins. For a recent review of sample preparation for protein analysis in LC-MS see Feist and Hummon [12]. The sensitivity for a specific peptide, natural or derived from a protein by enzymatic digestion with an enzyme such as trypsin, or protein will vary. However, a reasonable estimate of the difference on average in sensitivity analysed by a mass spectrometer for a peptide relative to an intact protein is in the range of 2-3 orders of magnitude more sensitive for peptide analysis.

You might have thought that immunoassays greatly preceded LC-MS for biomolecule detection and quantitation, but that would be incorrect. Mass spectrometry was

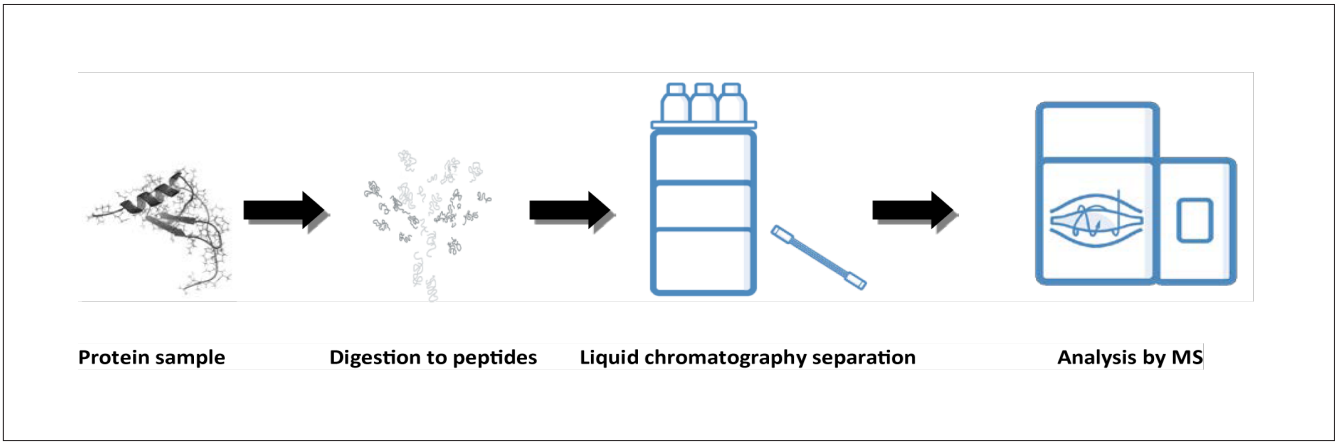

Figure 2. A typical LC-MS workflow. Proteins are extracted from the sample and either go through an enrichment/depletion step prior to digestion or are simply digested to peptides by a digestive enzyme. Peptides are then separated by HPLC, typically using a reverse-phase column, before analysis by mass spectrometry and peptides identified by appropriate software.

being used as early as 1964 for the detection and quantification of steroids [13]. Immunoassay, with its advantages at the time, became the gold standard, but over the past two decades significant advances in HPLC (high-performance liquid chromatography) and mass spectrometry have made LC-MS an attractive alternative to immunoassays. Indeed, an entire issue of Clinical Chemistry was recently devoted to LC-MS and its role in laboratory medicine. Annesley et al introduce that special issue [14]. In HPLC, advances in column technology to offer a wider range of chemistries and smaller particle sizes coupled with instrumentation capable of operating at higher pressures (smaller particle size in columns causes increased back pressure) and greater retention time precision has allowed for greater resolution and separation of analytes and faster and more reproducible separations. With mass spectrometry, technology advances have delivered greater performance as measured by metrics such as mass resolution, sensitivity and precision along with greater reproducibility, higher throughput and easier to use instrumentation [15]. A recent review by Jannetto and Fitzgerald gives an overview of the history and current application of mass spectrometry applied to the clinical laboratory [16]. Such technological advances have brought LC-MS in to sharp focus as a potential replacement or complementary technique for immunoassays.

Strengths of LC-MS

When evaluated, the limitations of immunoassays form the basis of many of the strengths of LC-MS:

- Selectivity – High resolution separation, coupled with mass spectrometry and in particular high resolution-accurate mass (HRAM) measurement by modern mass spectrometers allow for the identification and quantitation of biomolecules and even to distinguish between very structurally similar biomolecules and identification of PTMs. With LC-MS a new selective assay for multiple analytes can be developed in days while with immunoassays, as stated above, this typically takes several months for the generation of a specific antibody. LC-MS can suffer from interferences in an assay. However, these can typically be dealt with by amending either or both the chromatographic conditions to create separation of the analyte from the interference (for example changing the type of column) or by adjusting the MS/MS conditions.
- Sensitivity – Modern mass spectrometers are able to detect to the attomole level, and typically are more sensitive than immunoassay although there are exceptions. The caveat though is that to attain these attomole detection levels, effective sample preparation (for example sample extraction) and/or chromatography will need to be performed.
- Sample throughput – Using UHPLC (ultra high-performance liquid chromatography) allows for fast separation times, with less than five minutes achievable but depending on sample complexity. Analysis by mass spectrometry takes place concurrently with chromatographic separation adding no extra time. Automation also allows for the unattended running of thousands of samples. However each sample has to be run sequentially.
- Sample volumes – LC-MS typically uses low sample volumes (<5 μl). Thus microsampling can be used with LC-MS analysis, for example, with highly valuable samples or samples where it is not possible to obtain large volumes such as small animal models or from infants.
- Cost-per-sample – Most of the day-to-day running costs come from chromatography consumables which are fairly low, essentially consisting of the chromatography column and solvents. Typically a column will provide over 500 separations before it needs replacing and for UHPLC solvent requirements, volumes used are very low per sample (hundreds of microlitres per minute for several minutes).
- Reproducible – Intra and inter assay reproducibility is high with LC-MS. Retention time precision of modern HPLC instrumentation is extremely high with standard deviations typically less than 0.1% [17] and advancements in ionisation where sample is moved from liquid to gas phase and mass resolution have increased mass spectrometer measurement reproducibility. Inter lab reproducibility could still be improved, but with developments in protocols and electronic workflows this will happen.
- Multiplexing – This is undoubtedly a significant strength of LC-MS as the technology is inherently multiplexed in that many analytes can be analysed at a time for a single LC-MS analysis of a sample. Particularly with high resolution mass spectrometry approaches literally thousands of analytes can be measured, as is, for example, performed with proteomics and metabolomics experiments. In addition, compared to immunoassays, with LC-MS there is no increase in cost for analysing additional analytes while with immunoassays there is additional antibody cost with each additional target.
- Extended compound range – LC-MS is not limited to biomolecules and can identify and quantify a wide range of organic and inorganic compounds – as long

as a molecule can be ionised it can be detected. While the majority of compounds to be detected are likely to be biomolecules, although there is a role for LC-MS in therapeutic drug monitoring for example [18], LC-MS offers future-proofing for additional analytes that may not be biomolecules and/or when an antibody or affinity ligand cannot be generated against it.

Limitations of LC-MS

Undoubtedly there are many benefits to using LC-MS, but to date it has not displaced immunoassay which implies that LC-MS has or has had some limitations:

- Equipment costs – Undoubtedly one of the major obstacles to the widespread adoption of LC-MS technology in immunoassay laboratories is the initial cost of the equipment. A basic HPLC and very basic mass spectrometer system without MS/MS capability cost is on the order of one hundred thousand dollars and rises with more sophisticated and sensitive mass spectrometers to the hundreds of thousands. Coupled to this are the maintenance costs of the equipment. As pointed out above though, when the equipment is purchased the actual cost per sample is very low which spread over several years can actually result in overall cost reduction.
- Complexity – Mass spectrometry has been seen as complicated to operate and the resulting mass spectral data as difficult to interpret. This results in the need for trained staff which are in short supply and an investment in training for all staff involved in the operation of the mass spectrometer and analysis of data. Complexity has been reduced substantially over the past decade as instrumentation and software have become more user friendly and methods and applications have been developed and packaged in to more plug-and-play and sharable 'eWorkflows'. However, the perception of mass spectrometry as a difficult technology persists.
- Sample Complexity – Most samples presented for analysis are complex, plasma for example, with the dynamic range of proteins in plasma being enormous – one could be looking to quantify a protein that exists at only a few copies in plasma against an abundant protein such as albumin which is present in the billions and more. To put this in to further perspective, in plasma the difference between the highest and lowest abundance proteins is over 10 logs of molecular abundance and in most clinical assays over 4 logs [19]. With LC-MS, to identify and quantify a very low level protein, immunodepletion, for example, may have to be used to remove the more abundant proteins. Alternatively enrichment for lower abundance proteins or the use of more sophisticated UHPLC technology or multi-dimensional chromatography to increase protein/peptide separation prior to mass spectrometry might be needed. The issue of sample complexity, particularly in plasma, potentially can reduce the sensitivity of mass spectrometry which may need to be addressed with sample preparation.

Comparison of Immunoassays and LC-MS

Whilst immunoassays have been the gold standard for the past 50 years, especially in the diagnostics arena, that has not meant LC-MS has not been evaluated as an alternative to overcome some of the limitations of immunoassays. A number of comparison studies have been conducted to evaluate the performance of LC-MS against immunoassays, typically on analytes that give poor and conflicting results in immunoassays such as cortisol [20], glucagon [21] and vitamin D binding protein [22] to name but a few. The studies conducted to date generally show a good correlation between the two methodologies, even if the absolute concentrations differ due to the selectivity differences of both techniques. They do show however that LC-MS gives more accurate results and less false results which may be critical for treatment decisions, and, for example, for steroids significant multiplexed analysis is possible [23].

Will LC/LC-MS Ever Replace Immunoassays?

The immunoassay is a well established and routinely used technique, but as can be seen is affected by a number of flaws. Due to it being well established and a simple and cost effective assay to perform, we cannot envisage the replacement of immunoassays by LC or LC-MS in the very near future and there are a subset of simple, robust and routine assays that are likely to be applicable to immunoassays for the foreseeable future. However, technology is advancing and with the clear limitations inherent to immunoassays then LC and LC-MS will have a bigger role to play in the future since, particularly with LC-MS, more confidence in the accuracy of results is obtained. For LC and LC-MS to have a greater role though, the following needs to occur:

- Technology adoption – LC-MS is perceived as too complex and too expensive for most laboratories. Manufacturers have to demonstrate how they have reduced the complexity of operation and offer the required training to be competent. The lifetime cost of the instrumentation and the actual cost per sample needs to be clearly articulated. Finally, and most

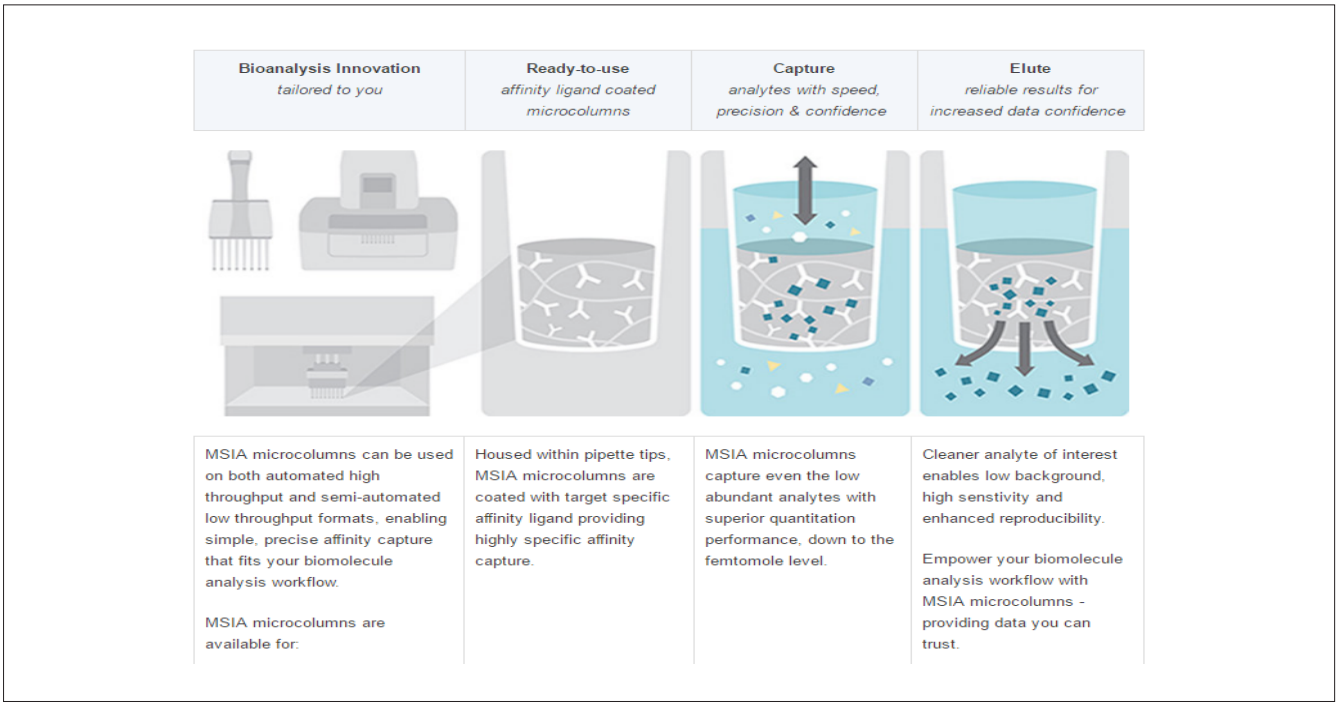

Figure 3. The Mass Spectrometric Immunoassay (MSIA) workflow. Image courtesy of Thermo Fisher Scientific.

importantly, it has to be clearly demonstrated that LC / LC-MS produces superior results and can do this in a more time and cost effective manner.

- Improvements in technology – Over the past decade dramatic improvements in LC-MS technology have been made to increase sensitivity, selectivity, reproducibility and robustness whilst also reducing the cost and complexity of the instruments. Complexity can be improved, or rather reduced, further by developing assays and protocols and automating these, with software performing the majority of the data analysis and interpretation. The most obvious technological improvement for the future would be to continue to increase the sensitivity of (multiplexed) detection and limit of quantification and/or improvements to sample preparation to aid sensitivity. Reproducibility is already reasonably high in LC-MS, but with developments in UHPLC retention time precision and resolution there is the exciting prospect that for some analytes with highly reproducible separations then the mass spectrometer could be dispensed with and replaced with less expensive UV detection only. This then further reduces the cost and complexity arguments.
- Assay development and validation – Assays currently conducted by immunoassay need to be transferred to and tested by LC-MS and ensure that the results from both platforms show good correlation. The assay then needs to be made in to kit format, amenable to automation and fully validated for use. Only when the alternative assays are developed, validated and shown to offer comparable or superior results, with more accuracy and precision and at less cost, and similar or greater throughput will the barriers to change be removed and LC-MS be broadly accepted.

Although these three elements are occurring, today one of the biggest barriers to full adoption of LC-MS is the issue of sample complexity for analysis of proteins. To address this researchers are typically using affinity chromatography to deplete high abundance proteins and/or enrich for the lower abundance proteins or peptides of interest. One such approach which combines some of the best elements of immunoassay with LC-MS is known as Mass Spectrometric Immunoassay (MSIA™) [24,25] which offers automatable, analytical affinity purification to capture and enrich low abundance proteins followed by elution for analysis by mass spectrometry. This represents a solution that could be implemented now to help adoption of LC-MS until the technology advances sufficiently to remove the requirement for this immuno-enrichment step.

Conclusion

The immunoassay has been the gold standard biomolecule assay for the past fifty years, but those fifty years

have highlighted both the benefits and limitations of the immunoassay. LC/LC-MS represents a complementary and potentially future replacement of the immunoassay by offering greater specificity, speed, analyte range, throughput and multiplexing capabilities coupled with a lower cost per sample and reduced sample volumes. For the fuller adoption of LC/LC-MS technology though further developments to the technology are needed. Importantly, communication of the above benefits of LC-MS need to be addressed to overcome the barriers to change from those currently using immunoassay, with fast, easy and cost effective sample preparation to reduce sample complexity also being key. Returning to the title of this review article; can LC/LC-MS ever replace immunoassays? Our current opinion is not yet. We believe that for some simple and routine assays then immunoassay will remain the most economical and simplest option, but for the majority of assays LC-MS is currently at least complementary if not superior to immunoassay and that the benefits LC-MS offers will see its increased use over immunoassay, especially as the technology advances. Longer term, the answer is yes.

References

1. Yalow RS, Berson SA. Assay of plasma insulin in human subjects by immunological methods. Nature 184 (Suppl. 21), 1648-1649 (1959).
2. Engvall E, Perlmann P. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry 8, 871-874 (1971).
3. Chang L, Li J, Wang L. Immuno-PCR: An ultrasensitive immunoassay for biomolecular detection. Anal Chim Acta 910, 12-24 (2016).
4. Rhyne PW, Wong OT, Zhang YJ, Weiner RS. Electrochemiluminescence in Bioanalysis. Bioanalysis 1(5), 919-935 (2009).
5. Nolan JP, Mandy F. Multiplexed and microparticle-based analyses: quantitative tools for the large scale analysis of biological systems. Cytometry A 69(5), 318-325 (2006).
6. Parashar A. Aptamers in therapeutics, J Clin Diagn Res 10(6), 1-6 (2016).
7. Spencer CA, Takeuchi M, Kazarosyan M, Wang CC, Guttler RB, Singer PA, Fatemi S, LoPresti JS, Nicoloff JT. Serum thyroglobulin autoantibodies: prevalence, influence on serum thyroglobulin measurement, and prognostic significance in patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab 83, 1121–1127 (1998).
8. Hoofnagle AN, Wener MH. The fundamental flaws of immunoassays and potential solutions using tandem mass spectrometry. J Immunol Methods 347(1-2), 3-11 (2009).
9. Rotmensch S, Cole LA. False diagnosis and needless therapy of presumed malignant disease in women with false-positive human chorionic gonadotropin concentrations. Lancet 355, 712–715 (2000).
10. Fuchs SM, Krajewski K, Baker RW, Miller VL, Strahl, BD. Influence of Combinatorial Histone Modifications on Antibody and Effector Protein Recognition. Curr Biol 21(1), 53-58 (2011).
11. Coucke W, Devleeschouwer N, Libeer J-C, Schiettecatte J, Martin M, Smitz J. Accuracy and reproducibility of automated estradiol-17β and progesterone assays using native serum samples: results obtained in the Belgian external assessment scheme. Hum Reprod 22(12), 3204-3209 (2007).
12. Feist P, Hummon AB Proteomic Challenges: Sample Preparation Techniques for Microgram-Quantity Protein Analysis from Biological Samples. Int J Mol Sci 16, 3537-3563 (2015).
13. Rhyage R. Use of a mass spectrometer as a detector and analyzer for effluent emerging from high temperature gas liquid chromatography columns. Anal Chem 36, 759-764 (1964).
14. Annesley TM, Cooks RG, Herold DA, Hoofnagle AN Clinical Mass Spectrometry – Achieving Prominence in Laboratory Medicine. Clin Chem 62(1), 1–3 (2016).
15. Eliuk S, Makarov A. Evolution of Orbitrap Mass Spectrometry Instrumentation. Annu Rev Anal Chem 8, 61-80 (2015).
16. Jannetto PJ, Fitzgerald RL. Effective use of mass spectrometry in the clinical laboratory. Clin Chem 62, 92–8 (2016).
17. De Pra M, Cook K, Oliver M, Paul C. Providing the Highest Retention Time and Peak Area Reproducibility for Maximal Confidence in Peptide Mapping Experiments. Thermo Scientific Application Note 1124 (2015).
18. McShane AJ, Bunch DR, Wang S. Therapeutic drug monitoring of immunosuppressants by liquid chromatography-mass spectrometry Clin Chim Acta 454, 1-5 (2016).
19. Hortin GL, Sviridov D. The dynamic rang eproblem in the analysis of the plasma proteome. J Proteomics 73(3), 629-636 (2010).
20. Monaghan PJ, Owen LJ, Trainer PJ, Brabant G, Keevil BG, Darby D. Comparison of serum cortisol measurement by immunoassay and liquid chromatography-tandem mass spectrometry in patients receiving the 11β-hydroxylase inhibitor metyrapone.

Annals of Clinical Biochemistry 48, 441-446 (2011).
21. Lapko VN, Miller PS, Brown GP, Islam R, Peters SK Sukovaty RL, Ruhn PF, Kafonek CJ. Sensitive glucagon quantification by immunochemical and LC-MS/MS methods. Bioanalysis 5(23), 2957-2972 (2013).
22. Denburg MR, Hoofnagle AN, Sayed S, Gupta J, de Boer IH, Appel LJ, Durazo-Arvizu R, Whitehead K, Feldman HI, Leonard MB. Comparison of Two ELISA Methods and Mass Spectrometry for Measurement of Vitamin D-Binding Protein: Implications for the Assessment of Bioavailable Vitamin D Concentrations Across Genotypes. J Bone Miner Res 31(6), 1128-1136 (2016).
23. Travers S, Martinerie L, Bouvattier C, Boileau P, Lombes M, Pussard E Multiplexed steroid profiling of gluco- and mineralocorticoids pathways using a liquid chromatography tandem mass spectrometry method. J Steroid Biochem Mol Biol, http://dx.doi.org/10.1016/j.jsbmb.2016.06.005 (2016).
24. Yassine H, Borges CR, Schaab MR, Billheimer D, Stump C, Reaven P, Lau SS, Nelson R. Mass spectrometric immunoassay and MRM as targeted MS-based quantitative approaches in biomarker development: potential applications to cardiovascular disease and diabetes. Proteomics Clin Appl 7(7-8), 528-540 (2013).
25. Peterman S, Niederkofler EE, Phillips DA, Krastins B, Kiernan UA, Tubbs KA, Nedelkov D, Prakash A, Vogelsang MS, Schoeder T, Couchman L, Taylor DR, Moniz CF, Vadali G, Byram G, Lopez MF. An automated, high-throughput method for targeted quantification of intact insulin and its therapeutic analogs in human serum of plasma coupling mass spectrometric immunoassay with high resolution and accurate mass detection (MSIA-HR/AM) Proteomics 14(12), 1445-56 (2014).

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