In this issue: Recent Developments in the Clinical Application of Mass Spectrometry

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Recent advances in the clinical application of mass spectrometry

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EDITORIAL

Since the latter half of the 20th century mass spectrometry (MS) applications, associated with gas chromatography (GC) separation (i.e. GC-MS), have been the "gold standard" in specialised clinical laboratories for the quantitation of drugs, organic acids and steroids [1]. This status quo remained unchallenged until just over a decade ago when liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and inductively coupled plasma mass spectrometry (ICP-MS) were introduced into routine clinical chemistry testing. This expansion and integration for many has been disruptive, but overall by and large, clinical chemistry laboratories are embracing MS for many analytes. This is exemplified by its increased presence in external quality assurance (EQA) programs [2,3,4,5]; Table 1 (see following page).

Today many clinical chemistry diagnostic laboratories have embraced MS, with electrospray ionization LC-MS/MS being the primary application. As such, there has been a rapid succession of methods in the peer reviewed literature which attest to their accuracy and precision. Whilst this technology clearly offers a number of significant advantages, including improvements in specificity and sensitivity, there is a dichotomous divide between advocates and detractors of MS based applications [6]; Table 2 (see table on page 269).

Table 1 Mass spectrometry based method principles reported for clinical chemistry analytes in the Royal College of Pathologists of Australasia (RCPA) Quality Assurance Programs (QAP)

Measurands included in the RCPAQAP Chemical Pathology Programs	Matrix	Program	Percentage of participants using MS method principle	Method principle
3-methoxytyramine	Plasma Urine	Plasma Metanephrines Urine Biogenic Amines	100% 58%	LC-MS/MS
4-hydroxy-3-methoxymethamphetamine (HMMA) / Vanillylmandelic Acid (VMA)	Urine	Urine Biogenic Amines	25%	LC-MS/MS
5-hydroxyindoleacetic Acid	Urine	Urine Biogenic Amines	27%	LC-MS/MS
17-hydroxy progesterone	Serum/Plasma	Endocrine	45%	LC-MS/MS
25-hydroxy vitamin D3	Serum/Plasma	Endocrine	10%	LC-MS/MS
Adrenaline	Urine	Urine Biogenic Amines	23%	LC-MS/MS
Aldosterone	Serum/Plasma	Endocrine	11%	LC-MS/MS
Aluminium	Serum Urine	Trace Elements	62% 83%	ICP-MS
Amiodarone	Serum/Plasma	Special Therapeutic Drugs & Antibiotics	25%	LC-MS/MS
Androstenedione	Serum/Plasma	Endocrine	44%	LC-MS/MS
Arsenic	Urine Whole blood	Trace Elements	90% 88%	ICP-MS
Benzodiazapines e.g. Oxazepam	Urine	Urine Toxicology	30%	GC-MS (11%), LC-MS/MS (14%), LC-TOF/MS (5%)
Cadmium	Urine Whole blood	Trace Elements	83% 83%	ICP-MS

Chromium	Serum Urine	Trace Elements	78% 80%	ICP-MS
Clozapine	Serum/Plasma	Special Therapeutic Drugs & Antibiotics	23%	LC-MS/MS
Cobalt	Serum Urine	Trace Elements	100% 90%	ICP-MS
Copper	Serum Urine	Trace Elements	39% 60%	ICP-MS
Cortisol	Saliva Serum/Plasma	Salivary Cortisol Endocrine	19% 2%	LC-MS/MS
Cyclosporin	Serum/Plasma/ whole blood	Special Therapeutic Drugs & Antibiotics	13%	LC-MS/MS
DHEAS	Serum/Plasma	Endocrine	5%	LC-MS/MS
Dihydrotestosterone	Serum/Plasma	Endocrine	63%	GC-MS (13%), LC-MS/MS (50%)
Dopamine	Urine	Urine Biogenic Amines	24%	LC-MS/MS
Homocysteine	Serum/Plasma	Endocrine	2%	LC-MS/MS
Homovanillic acid (HVA)	Urine	Urine Biogenic Amines	23%	LC-MS/MS
IGF-1	Serum/Plasma	IGF-1 / C-peptide	3%	LC-TOF/MS
Iodine	Urine	Trace Elements	89%	ICP-MS
Lead	Urine Whole blood	Trace Elements	77% 48%	ICP-MS
Manganese	Serum Urine Whole blood	Trace Elements	100% 88% 78%	ICP-MS
Mercury	Urine Whole blood	Trace Elements	100% 100%	ICP-MS

Metanephrine	Urine	Urine Biogenic Amines	48%	LC-MS/MS
Mycophenolate	Serum/Plasma	Special Therapeutic Drugs & Antibiotics	33%	LC-MS/MS
Nickle	Urine	Trace Elements	89%	ICP-MS
Noradrenaline	Urine	Urine Biogenic Amines	21%	LC-MS/MS
Normetanephrine	Urine	Urine Biogenic Amines	48%	LC-MS/MS
Oestradiol	Serum/Plasma	Endocrine	1%	LC-MS/MS
Plasma free metanephrine	Plasma	Plasma Metanephrines	93%	LC-MS/MS
Plasma free normetanephrine	Plasma	Plasma Metanephrines	93%	LC-MS/MS
Progesterone	Serum/Plasma	Endocrine	1%	LC-MS/MS
Selenium	Serum	Trace Elements	82%	ICP-MS
	Urine		100%	
	Whole blood		83%	
Serotonin	Urine	Urine Biogenic Amines	50%	LC-MS/MS
Sirolimus	Serum/Plasma/ whole blood	Special Therapeutic Drugs & Antibiotics	38%	LC-MS/MS
Sweat Chloride	Sweat	Sweat Electrolytes	24%	ICP-MS
Tacrolimus	Serum/Plasma/ whole blood	Special Therapeutic Drugs & Antibiotics	17%	LC-MS/MS
Testosterone	Serum/Plasma	Endocrine	9%	LC-MS/MS
Thallium	Urine	Trace Elements	100%	ICP-MS
Tricyclic antidepressant general screen	Serum/Plasma	Special Therapeutic Drugs & Antibiotics	13%	LC-TOF/MS
Vanadium	Urine	Trace Elements	67%	ICP-MS
Vitamin A (retinol)	Serum/Plasma	Vitamins	3%	LC-MS/MS
Vitamin B1 (thiamine pyrophosphate)	Whole blood	Vitamins	4%	LC-MS/MS
Vitamin B6	Serum/Plasma	Vitamins	17%	LC-MS/MS

Zinc	Serum Urine Whole blood	Trace Elements	39% 80% 67%	ICP-MS

The percentage of mass spectrometric methods reported is based on the latest end of cycle or interim reports available on the RCPAQAP website. This data is presented with permission from the RCPAQAP Chemical Pathology Programs

In addition, there is a clear and real problem of finding staff equipped with the dual skills of MS and laboratory quality management. Hence, we need to look for new education and training approaches for emerging and current medical scientists/technologists that accommodate for these prerequisites. This will support the use of MS within a quality framework, enabling us to continue to meet expectations of MS as the "gold standard" method.

In this issue of the *eJournal of the International Federation of Clinical Chemistry and Laboratory Medicine,* there are four articles which highlight the changing landscape of MS based applications [7,8,9,10]. Together these explore changes and advances to instrumentation which paves the way for new approaches. The opening manuscript by Mbughuni and colleagues provides a clear overview of the range of current and emerging MS technologies available; which is driven in part by the significant need for the toxicology laboratory to keep abreast of illicit drugs and challenges of detection and quantitation [7]. Mbughuni further explores the matrices available for drug analysis which includes the use of dried blood spots. Following on from this article a detailed review of the extensive application of dried blood spot MS analysis, for analytes outside of new born screening applications, is provided by Zakaria and colleagues [8]. Then Kam and colleagues explores the emerging applications of peptide quantification by MS, taking a specific look at insulin-like growth factor I (IGF-I) [9]. Finally, in the last article of the special edition, Dias and Koal explore the future of MS in the clinical laboratory through the progress of standardisation in metabolomics and its potential role in laboratory medicine [10].

Together these manuscripts highlight the challenges and importance of quality management principles to achieve results that are fit for their intended clinical purpose. There are five recognised pillars supporting standardisation; certified reference materials (CRM), reference measurement procedures (RMP), reference laboratories, reference intervals or decision points and participation in an external quality assurance program. Information on the first three pillars is provided in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database [11]; currently some (e.g. serum testosterone) but not all measurands (e.g. dried blood spot analytes) measured by mass spectrometry have complete listings, indicating deficiencies in the traceability chain [11]. As we continue to embrace MS technology, it is important that we also concentrate on developing and implementing these five important pillars to ensure that standardisation with traceability is achieved.

Participation in an EQA program is recognised as the central pillar supporting harmonisation of methods [12]. Such harmonisation is not however necessarily true for these newer applications which do not yet have robust EQA programs available or the critical number of laboratories for this comparison to occur. This is particularly highlighted in the discussion from

Table 2 Five points and counterpoints why laboratories are reticent to introduce LC-MS/MS. Points of detractions are provided from an online social media blog. Counterpoints are provided by the author (RG)

No.	Point of detraction [6]	Counterpoint
1	"Mass Spec is Too Complicated"	Quality Management (QM) is also complicated. A director of a large laboratory said "It is easier to train a diagnostic laboratory scientist in MS, as they understand the background, than to take someone from e.g. a research background with MS experience and train them in pathology" [anonymous personal communication].
2	"Mass Specs Are Too Big"	But many of our automated analysers are also large.
3	"Too Expensive"	Agree MS does seem expensive, but this is because we are use to reagent rental agreements from some immunoassay companies. It is important to create a business case to demonstrate return on investment.
4	"Testing Takes Too Long"	This is currently usually true, but will probably change in the future as MS becomes more automated.
5	"We use GC-MS/MS, and it Works Fine"	There is still an important place for GC-MS or GC-MS/MS in the laboratory, but the advantage of LC-MS/MS is that derivatisation is not mandatory. In addition, GC-MS or MS/MS has a clear role in discovery applications as highlighted by Dias and Koal [10].

Kam and colleagues related to the measurement of peptides by MS. Whilst there are EQA schemes available for IGF-1, participation is currently predominated by immunoassay methods and medians are often used to assess performance [2]. In the absence of a CRM and RMP robust EQA target values cannot be developed to aid the determination of bias for the small number of MS participants. However, there is still some value in participation in an EQA program (such as the RCPAQAP) as imprecision and linearity can be determined statistically and participation encourages other MS users to join to create the critical numbers. When an EQA program is not available sample exchange should be given high priority to support both method validation and on-going harmonisation of MS methods.

Sample exchange and/or EQA participation is often the first step in the recognition of discordance between results. A number of studies have demonstrated that there are factors independent from the choice of calibrator that can cause variation in MS results [13,14,15,16]. Whilst the authors in this special edition have drawn our attention to a number of important considerations, there is little discussion related to the choice of isotope selected for use as the internal standard and how this can influence the quantitation of results [7,8,9,10]. A two deuterated (D) internal standard is generally

not recommended where there are reasonable alternatives, as it is only two additional daltons from the target analyte which may lead to interference at high concentrations due to the presence of 13C2 isotopomers of the target [15,17,18]. A study by Owen and colleagues, comparing three internal standards (D2, D5 and C13) for serum testosterone quantitation by LC-MS/MS, demonstrates the influence of internal standard choice on patient results [16]. In addition, a study by Flynn and colleagues for the quantitation of epi-25 hydroxy vitamin D3 highlights the need for internal standards to co-elute with the compound of interest so they are present in the ion source at the same time. Hence attention is required for the appropriate selection of the internal standard for accurate quantitation of LC-MS/MS measurands and to achieve harmonisation of the current and future methods [17,19].

A contemporary challenge exists in relation to the amount of data generated from the MS. Interpretation of results against a reference interval or clinical decision point is critical to turn the numerical result into a clinically meaningful result. This is the challenge for many current MS assays and also the newer methods discussed in this edition of the journal [7,8,9,10]. In particular, the metabolomics discussion by Dias and Koal illustrates the need to develop an additional skill set of statistical analysis and/or employ statisticians to support the analysis of the magnitude of data generated in these MS discovery applications [10].

In conclusion, MS is now firmly established in the clinical space and the range of applications will continue to expand. Whilst MS is not yet applicable for all regions, in the future just like the manual immunoassays of old, MS throughput and user friendliness will improve. As we embrace MS our current and future scientists ideally should have the combined skills to 1) validate and run the current and new clinical MS applications, 2) work within a quality framework and 3) apply appropriate statistical analysis for the interpretation of the data. Developing scientists with these combined skills will support the robustness of methods, goals of harmonisation and eventual standardisation with traceability of MS methods.

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