Opinion

A Guide to Harmonisation and Standardisation of Measurands Determined by Liquid Chromatography – Tandem Mass Spectrometry in Routine Clinical Biochemistry

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Abstract
Globally, harmonisation in laboratory medicine is a significant project. The relatively new implementation of liquid chromatography coupled with tandem mass spectrometry (LC-MSMS) techniques as routine assays in diagnostic laboratories provides the unique opportunity to harmonise, and in many cases standardise, methods from an early stage. This guide aims to provide a practical overview of the steps required to achieve agreement between LC-MSMS analytical procedures for routine clinical biochemistry diagnostic assays, with particular focus on the harmonisation and standardisation of methods currently implemented.

To achieve harmonisation, and where practical standardisation, the approach is more efficient if divided into sequential stages. The suggested division entails: (i) planning and preliminary work; (ii) initial assessment of performance; (iii) standardisation and harmonisation initiative; (iv) establishing common reference intervals and critical limits; (v) developing best practice guidelines; and (vi) performing an ongoing review.

The profession has a unique and significant opportunity to bring clinical mass spectrometry-based assays into agreement. Harmonisation of assays should ultimately provide the same result and interpretation for a given patient's sample, irrespective of the laboratory that produced the result. To achieve this goal, we need to agree on the best practice LC-MSMS methods for use in routine clinical measurement.

Introduction

It is clear that the mission of each clinical laboratory is to provide the *right result* on the *right patient* at the *right time*. With the introduction of the European Union In Vitro Diagnostic Medical Devices Directive,[1] the *right result* must be traceable to a higher order measurement procedure and/or higher order reference material. This directive has provided significant impetus to solve the problem of poor inter-laboratory agreement of results for measurands across methods.

The concept of inter-laboratory agreement, or harmonisation, for measurands of clinical importance is not in itself a new process. Probably the most successful harmonisation project, first suggested in 1967, rationalised the procedures for prothrombin time measurement,[2] leading to the International Normalised Ratio (INR),[3,4] a model still in world-wide use today.[5]

Globally, harmonisation in laboratory medicine is a significant project, currently directed towards established

high throughput, automated clinical biochemistry assays.[6] This process potentially requires changes to established procedures, methods, reference materials, reference intervals and/or clinical decision points. It also involves co-operation and consensus agreement among stakeholders, metrology organisations, in vitro diagnostic companies, laboratories and clinicians. The relatively recent implementation of liquid chromatography (LC-MSMS) techniques for routine assays in diagnostic laboratories provides the unique opportunity to harmonise, and in many cases standardise, these methods from the outset.

This guide aims to provide a practical overview of the steps required to achieve agreement for LC-MSMS analytical procedures for routine clinical biochemistry diagnostic assays, with particular focus on the harmonisation and standardisation of methods currently implemented. There are a number of key terms that are regularly incorporated into discussions related to global harmonisation in laboratory medicine. These terms include a full definition of the 'measurand' and its 'uncertainty of measurement', 'harmonisation', 'standardisation', 'calibration', 'traceability', 'commutability' and 'fitness for purpose'. An understanding of terminology is crucial as all stakeholders work together to achieve harmonisation in laboratory medicine. A detailed description of each of these terms is given in the Table.

Mass Spectrometry in the Clinical Biochemistry Laboratory

While the early pioneering work of Thompson laid the foundation for the technical development of mass spectrometry as an analytical tool,[7] it was not until the 1950s that gas chromatography (GC) was successfully coupled to mass spectrometric (MS) detection.[8] By the 1980s GCMS systems were being used in clinical biochemistry laboratories for the detection of drugs, organic acids and steroids.[9] This specialised technique is limited by the requirement to make analytes volatile, usually by chemical derivatisation, and by the compound's ability to withstand temperatures up to about 300°C. For measurands suitable for analysis by GCMS, this technique has become a gold standard method for many and is an important component of method standardisation.[10]

The 'development of soft desorption ionisation methods for mass spectrometry analysis of biological macromolecules' realised the wider sample application of mass spectrometry.[11] Fenn's development of electrospray ionisation in the 1980s saw manufacturers begin to develop liquid chromatography mass spectrometry systems, allowing non-volatile, non-derivatised samples to be analysed. The introduction of LC-MSMS systems into Australian routine clinical laboratories began with newborn screening in the 1990s. There followed therapeutic drugs, drugs of abuse and toxicology, vitamin D, steroids, biogenic amines, calibration and reference data comparison for measurands such as creatinine, and peptide analysis.

Currently, the adoption of LC-MSMS systems and implementation of methods appear to differ between countries as indicated by participation in external quality assurance (EQA) programs, a mandatory requirement for clinical diagnostic laboratories.[12] For example, comparing reported method principles between the Royal College of Pathologists of Australasia (RCPA) Quality Assurance Programs Pty Ltd (QAP) and the United Kingdom's National External Quality Assurance Scheme (UKNEQAS) for urinary free cortisol, at the start of 2012, no participants in the QAP reported using LC-MSMS for this measurand (12% reported using LC without MS detection) whilst 24% of participants in UKNEQAS used LC-MSMS.[13,14]

Advantages and Challenges of LC-MSMS

Specificity of LC-MSMS analysis has been one of the prime motivators in any decision to change to this technique. In this regard, it is superior to immunoassay in which lack of antibody specificity may result in significant cross-reactivity of related compounds (both metabolically active and inactive), possibly confounding the interpretation of the result.[15,16] The ability to quantify a measurand accurately by MSMS using multiple reaction monitoring (isolation of a precursor and a product ion) provides greater specificity (Figure 1). However, the LC separation of structurally related isomers (and isobaric compounds) is essential to achieve optimal specificity, as such related compounds have identical precursor and product ions and cannot be distinguished in the tandem mass spectrometer. Well-publicised problematic measurands (and their associated methods) include 25-hydroxyvitamin D3 separation from epi-25-hydroxyvitamin D3,[17] testosterone separation from epi-testosterone,[18] and structurally related isomers seen in urine steroid metabolomic profiles.[19] Nevertheless, combining appropriate LC separation with MSMS detection provides specificity that is unmatched by current immunoassay techniques.

With the advantage of simultaneous detection of multiple analytes (i.e. multiplexing) in the MSMS comes the problem of alterations in ion formation i.e. ion suppression or enhancement. Ion suppression is the decrease in signal of a compound when present in a mixture rather than in pure solution at the same concentration.[20,21] External standardisation is therefore not appropriate. The development and use of structurally identical stable isotopes as internal standards minimises the problem of ion suppression (or ion

Table. Working definitions of key terms associated with the concepts of harmonisation and standardisation.*

Measurand. The International Vocabulary of Metrology (VIM) defines measurand as the 'quantity intended to be measured'. Often in laboratory medicine this term is erroneously replaced with the less specific term 'analyte'.[39]

Harmonisation. Harmonisation is the process of making agreement between methods in order to produce a consistent clinical interpretation irrespective of the laboratory in which samples are analysed. The outcome of harmonisation does not necessarily equate to 'trueness'. Harmonisation is commonly used to describe either of the following two processes:

- Harmonisation of the overall testing process, encompassing the pre-analytical phase, methods of analysis, calibration materials, units of reporting and reference intervals. Harmonisation in this broader sense can be applied to the critical aspects that should be aligned to promote agreement. The development of implementation guidelines and best practice statements form part of this process.
- Harmonisation of calibration material. When the term harmonisation (rather than standardisation) is used, it may mean that no primary reference material or higher order reference measurement procedure exists and values may not be traceable to the International System (SI) of units.[40] It may also indicate that the measurand cannot be clearly defined in terms of molecular mass and SI units; therefore currently standardisation is not achievable and agreement must be achieved through harmonisation, in which case trueness is not established.

Standardisation. Standardisation is a concept whereby agreement of test results is achieved by establishing traceability to higher order reference materials and measurement procedures.[41] This is achievable when the method base and the measurand can be clearly defined. The measurand cortisol is one example where standardisation is achievable as the molecule is clearly defined in terms of molecular mass; hence the calibration process to achieve uniformity of cortisol results can be regarded as standardisation. The outcome of standardisation is agreement with established trueness.

Calibration. An 'operation that, under specified conditions….establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties'.[39]

Traceability. Metrological traceability is defined by VIM as the 'property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement of uncertainty'. Such traceability requires an established hierarchy. The Joint Committee for Traceability in Laboratory Medicine (JCTLM) was founded in 2002 to establish this hierarchy through the development of reference libraries to recognise the higher order reference methods, certified reference materials and reference laboratories worldwide. Their database provides a significant resource to realise and support worldwide reliable comparability and traceability of measurement results in laboratory medicine.[42]

Measurement Uncertainty. A "non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used".[39] There are many components to uncertainty including the finite detail in the definition of a measurand, the inaccuracy of a calibrator's assigned value and the imprecision associated with the method. VIM separately defines .

- Type A as the "evaluation of a component of measurement uncertainty by a statistical analysis of measured quantity values obtained under defined measurement conditions".[39]
- Type B as the "evaluation of a component of measurement uncertainty determined by means other than a Type A evaluation of measurement uncertainty".[39]

Type A uncertainty, which defines analytical imprecision in relation to the 95% confidence interval of a result, is routinely estimated by ISO15189 accredited laboratories.[12] In contrast, Type B uncertainty (which relates to the accuracy of measurement with reference to traceability, certified reference materials and measurement instruments) is usually estimated by the manufacturer of the calibrator and included as part of the certificate of analysis.

Commutability. Commutability was first used to describe the 'ability of an enzyme material to show inter-assay activity changes comparable to those of the same enzyme in human serum'.[43] The Clinical and Laboratory Standards Institute provides a practical definition to encompass all measurands/analytes specific to laboratory medicine being:

- The 'property of a reference material, demonstrated by the equivalence of the mathematical relationships among the results of different measurement procedures for a reference material and for representative samples of the type intended to be measured'.[44]

Commutability needs to be established experimentally to ensure that calibrators, patient samples and external quality assurance (EQA) materials are comparable between methods. Establishing the commutability of a reference material for a specific measurand confirms that it may be used as a calibrator. [22]

Fitness for purpose. A description used to express the appropriateness of a clinical method in relation to biological variation, and clinical decision requirements.[45] Fitness for purpose is described for quantitative methods as 'optimal', 'desirable' or 'minimum',[46] based on the analytical imprecision of a method compared with the intra-individual biological variation expected in a patient's result.[47] To achieve 'optimal', 'desirable' or 'minimum' fitness for purpose the analytical imprecision must be less than or equal to 25%, 50% or 75% of the intra-individual biological variation respectively.[46] For most, but not all, clinical biochemistry assays, fitness for purpose to at least the 'minimum' standard is achievable.

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*Defining the measurand is essential to the processes of harmonisation and standardisation.

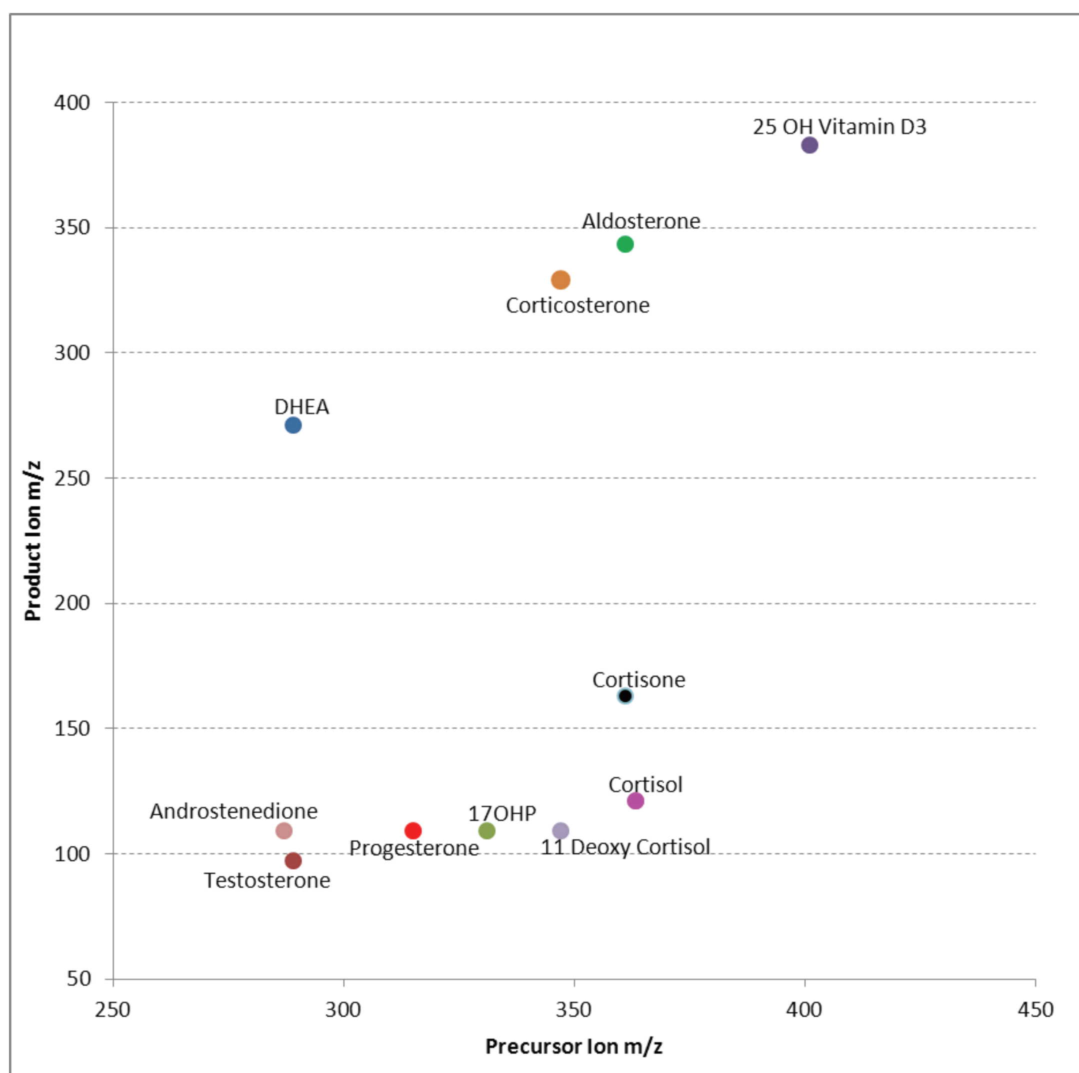

Figure 1. LC-MSMS detection of steroid analytes. The selection of each of these precursor and product ions is a significant component of specificity. DHEA = dehydroepiandrosterone; 17OHP = 17-hydroxyprogesterone; m/z = mass to charge ratio.

enhancement) as similar alterations in ion formation are seen in the internal standard as in the target measurand. Apart from its interference in quantification, ion suppression is a significant contributor to poorer than expected sensitivity.[20]

The sensitivity of LC-MSMS systems as determined by signal to noise ratios has significantly improved in recent times. Whilst the precursor ion is normally associated with the molecular mass of the compound, the product ions are chosen from the fragmentation ions. One product ion is selected for quantification, whilst a second and sometimes third product ion is selected as the qualifier ion to further ensure analytical specificity. The signal to noise ratio of the selected quantifier and qualifier product ions will determine the overall sensitivity of the measurand.

Multipoint calibration of LC-MSMS systems is common practice. Laboratories currently either prepare their standards in-house by gravimetrically weighing in pure substances, or purchase secondary calibration material when available. Because such materials are commonly not identical to patient samples in terms of their matrix, commutability should be verified.[22] The standards used for calibrating LC-MSMS systems should be traceable to certified reference materials[10] which achieve a higher accuracy and lower uncertainty compared with equivalent secondary calibrators.[23]

Defining the LC-MSMS Method

When LC-MSMS methods were first introduced into the routine clinical laboratory, it was generally presumed that accuracy was assured. Reality demonstrated that for the same measurand, bias and imprecision of methods (despite their relative specificity) were present both within and between laboratories. One prominent example is the analysis of the immunosuppressant tacrolimus, where the need for standardisation has been highlighted in order to improve its clinical utility.[24] Inconsistencies associated with calibrator selection, poor chromatographic separation, inappropriate analyte recovery, ion suppression and selection of inappropriate product ions contribute to poor inter-laboratory agreement.

Each method introduced into a laboratory's repertoire (whether LC-MSMS or alternative) requires an understanding of its performance characteristics and limitations. To do this, method validation experiments need to be conducted. Studies to validate methods include linearity, sensitivity,[25] bias, imprecision (uncertainty), recovery,[26] interference, stability and sample carry-over. Additional studies are required for LC-MSMS assays to confirm ions and ion ratios for quantification.[27] Method validation guidelines have been developed to cover all quantitative assays including MS along with specific guidelines for small molecules analysed by LC-MSMS in the routine diagnostic laboratory.[27-29]

Documenting specific acceptability criteria in advance of method validation studies is good practice.[27] However, the acceptable performance of routine and reference LC-MSMS methods is not clearly defined. Currently, acceptable performance for trueness and imprecision of routine LC-MSMS methods is generally based on peer performance and clinical requirements. The work of Stöckl and colleagues with 25-hydroxyvitamin D provides a guide to establishing specifications for both routine and reference LC-MSMS methods for other measurands.[30] Understanding the boundaries of the method through method validation studies, defined acceptability criteria and their relationship to biological variation and clinical needs is paramount for good laboratory practice and essential background work in the harmonisation process.

The harmonisation, or agreement, of methods is of high importance for the interpretation of results using common reference intervals.[31] With LC-MSMS methods, the measurand may be significantly different from that obtained by immunoassay. Even when the methods use common calibrators that are truly commutable and traceable to higher order reference methods or materials, the final results may still differ. If this is the case, the approach of transference of reference intervals[32] cannot be applied and the need to generate specific MS-derived reference intervals which are distinct from immunoassay-specific reference intervals is likely. This in itself is a significant undertaking, especially when determination of the reference interval may be required for many different groups. For example, stratification by age, gender and menstrual cycle stage would be required for oestradiol.

In the era of evidence-based laboratory medicine and the increasing implementation of LC-MSMS methods, achieving harmonisation with associated common reference intervals will provide: the uniformity required to ensure clinicians make the appropriate interpretation of results; for clinical societies to generate diagnostic and treatment guidelines; and for patients to have the flexibility of follow-up testing performed by various laboratories.

Practical Approach To Harmonisation of Routine Laboratory LC-MSMS Methods

The areas in clinical biochemistry relevant to LC-MSMS where harmonisation is considered to be highly desirable are identified as newborn screening, inborn errors of metabolism, immunosuppressants, steroids and vitamins, especially vitamin D.[33,34] The metrological traceability

approach aims to provide true and precise routine assays.[35] To achieve harmonisation, and where practical standardisation, the approach is more efficient if divided into sequential stages. The suggested division entails: (i) planning and preliminary work; (ii) initial assessment of performance; (iii) standardisation and harmonisation initiative; (iv) establishing common reference intervals and critical limits; (v) developing best practice guidelines; and (vi) performing an ongoing review. This staging process is presented diagrammatically in Figure 2.

Stage One – Planning and Preliminary Work

As with all development work, the success of the final outcome is dependent on the initial planning stage. Careful consideration should be given to the choice of measurand(s) to harmonise, establishment of project goals and a project plan to achieve these goals, plus recruitment of active member laboratories.

Identify and Prioritise Measurand(s)

Selection of measurands with a perceived high clinical need for harmonisation is often based on the expert but somewhat subjective assessment of members initiating the harmonisation project. The initial aim should be to 'keep it simple' with the selection of one measurand rather than a group of common measurands. In this way goals developed are likely to be more readily achieved.

Form a Project Group – Recruitment of Laboratories

As there is still only a limited number of routine laboratories

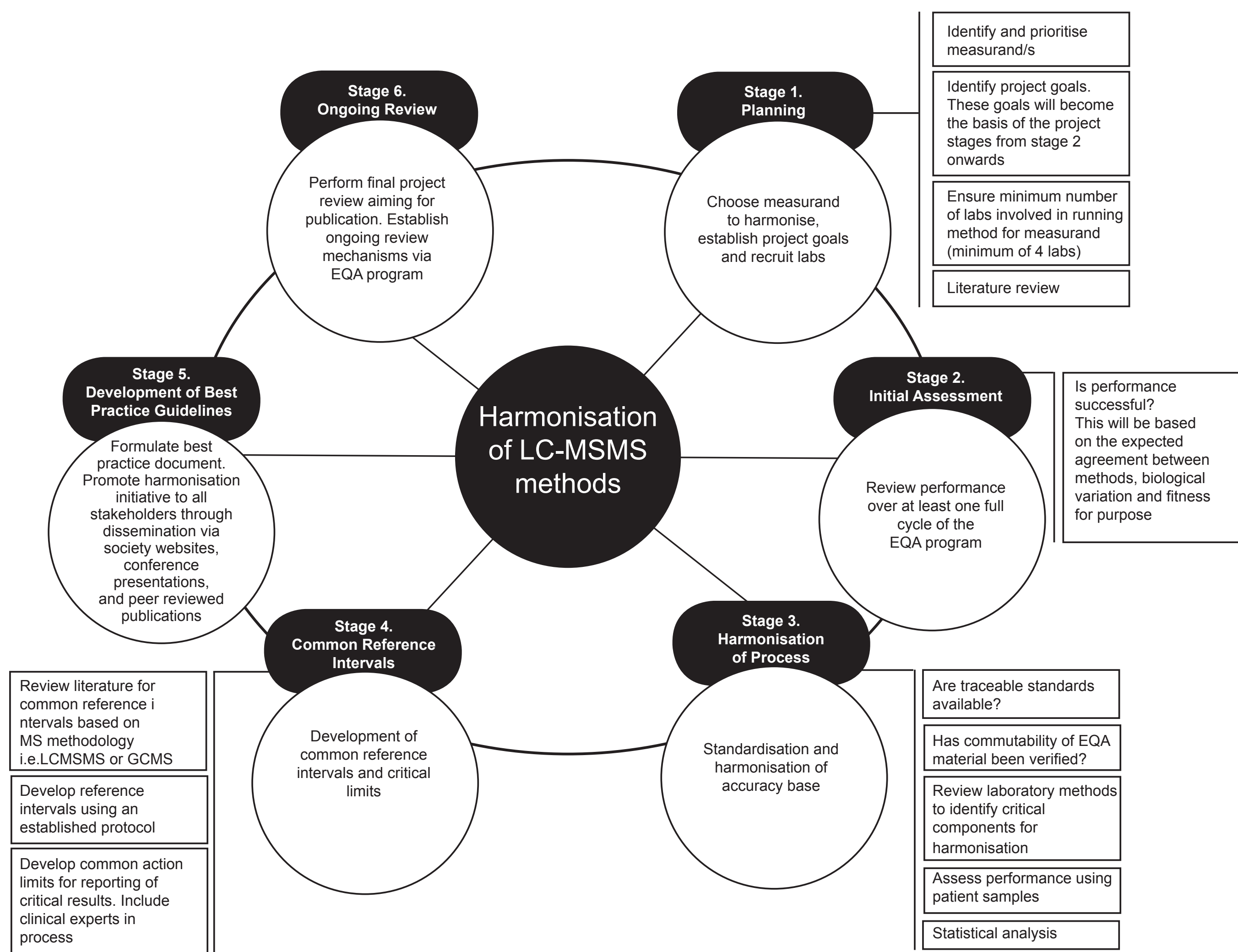

Figure 2. Mindmap of practical steps to achieve standardisation and overall harmonisation when the measurand can be defined.

running methods for all the LC-MSMS measurands identified for harmonisation, the overall number of participants will be relatively small. An absolute minimum of four laboratories should be involved in running the relevant methods for harmonisation although a higher number would improve the robustness of the harmonisation process.

Establish Project Goals

It is important to identify the project goals from the outset to provide clear direction. These goals will become the basis of the project stages from stage two onwards. Examples of project goals include:

- to provide detailed method information on the measurand of interest in the form of a best practice statement
- to develop standard operating procedures including use of a commutable material as a common calibrator
- to harmonise reference intervals

Project Documentation

For each project, the group should prepare a document to define the project. Suggested inclusions are: definition of the project; goals; strategies to achieve goals; and timeline.

External Quality Assurance Program

The participation of project members in a common EQA program allows comparison of results between members. As such, members of the one project group should agree to participate in the same EQA program for the life of the project. Ideally the selected scheme should have targets set by reference measurement systems rather than using the median of participants' results. An inventory of external Quality Assurances Schemes offered worldwide is provided by the Centers for Disease Control and Prevention (CDC).[36]

Stage Two – Initial Assessment of Performance

To effectively compare analytical methods, whether LC-MSMS or other, it is good practice to collate information for assessment of performance.

Method Registration

Develop a detailed method registration document for participants to register their initial method. This document should provide a comparison for the individual components of each method in order to identify common factors and areas where discordance exists. Important components of the method registration document include:

- calibrators and their traceability
- internal standards selected and their source
- sample extraction process and recovery
- components of LC analysis i.e. column, mobile phases, injection volume and run time
- brand of mass spectrometer and ion source
- precursor and product ions for quantification
- qualifier ions
- method validation parameters such as linearity and sensitivity
- reference intervals.

Participation in an EQA Program

Review performance over at least one full cycle of the selected EQA program. This should include acceptable performance in terms of bias and imprecision. The EQA program should ideally have their measurand target values set through analysis by a higher order reference method employing a certified reference material (CRM) rather than relying on group medians.

Compare Assessment of EQA Performance Against Patient Samples

To ensure the performance characteristics observed with the EQA samples are similar to those seen with patient samples, a set of de-identified patient samples should be sent to each project laboratory for LC-MSMS analysis of the measurand of interest. Between-laboratory imprecision can be compared with the between-laboratory imprecision of the EQA samples. Bias can also be compared when both the patient samples and the EQA material have values assigned by a reference laboratory. The determination of an acceptable comparison may be based either on statistical significance or clinical decision limits.

Stage Three – Standardisation and Harmonisation Initiative

From stage two, an understanding of initial performance is gained. This information should be used to make informed decisions related to standardisation and harmonisation requirements.

Standardisation Process

Achieving calibrator standardisation with traceability is a significant factor towards achieving the harmonisation of patient values. If commercial secondary calibrators traceable to CRMs are available, these secondary calibrators should be assessed for acceptable concentration levels for the clinical samples of interest, stability and commutability. If there are no commercial secondary calibrators traceable to a CRM available, the following may be considered:

- Liaise with a metrology institute to develop a primary reference material if none is identifiable from the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database. Whilst this is the ideal approach and is based on the principle of standardisation, it is also expensive and initially costs may be prohibitive.
- Identify an appropriate secondary calibrator for assessment of commutability between routine LC-

MSMS methods. When traceability has not been predetermined, liaise with a metrology institute to assign the secondary calibrator values against a CRM.
- Identify a secondary calibrator that is commutable with patient samples between routine LC-MSMS methods and assign values based on a group consensus process. Although this approach does not provide traceability or guarantee trueness, it does enable calibration agreement between laboratories for the harmonisation process.

Harmonisation of Methods
Review agreement of EQA and patient results after the implementation of the common calibrator. If the overall intra- and inter-laboratory method bias and imprecision fall outside the defined project group specifications then further method agreement is required. This can be achieved by review of the method registration documents completed in stage two and also by reference to higher order LC-MSMS reference measurement systems listed on the JCTLM database.

Inclusion of GCMS Analysis in Method Evaluation
For measurands that are suitable for GCMS (or GC-MSMS) analysis, a method comparison should be performed between the GCMS and LC-MSMS methods to ensure that the results are commutable. The inclusion of GCMS analysis provides an additional quality assurance step as both measurement systems have their strengths and limitations.

Review
The acceptable bias and imprecision of the measurement procedures should be reviewed at this point to assess whether the calibrator and method harmonisation strategies have been successful.

Stage Four – Common Reference Intervals and Critical Limits
With standardisation and harmonisation, common reference intervals can be applied between laboratories, and results from one laboratory can be directly compared with another. This ultimately improves patient care.

Review the Literature
Review the literature for common reference intervals based on MS methodologies i.e. LC-MSMS or GCMS. If such reference intervals are found then it may be possible to transfer these intervals to the harmonised methods using the transference of reference interval approach.[32]

Development of Reference Intervals
If published LC-MSMS reference intervals are not available or cannot be transferred, then common LC-MSMS reference intervals and also critical limits will need to be developed. This will require a minimum of 120 participants for each stratification (such as age and gender) of the population of interest.[37]

Determine Critical Limits
In consultation with clinical staff, determine critical limits for urgent reporting of results.

Stage Five – Best Practice Guidelines
To promote the harmonisation initiative to all stakeholders, the formulation of a best practice document is essential.[38] The method registration document developed in stage two may be valuable as the starting point, and a recommended method should be included as part of this best practice document.

Stage Six – Ongoing Review
At this stage, harmonisation of LC-MSMS methods, and where practicable standardisation, of the project measurand has been achieved. A final project summary should be conducted to assess the outcomes against the initial project goals. In addition a mechanism for an ongoing review via the EQA program should be established to ensure continued harmonisation.

To date, applications in the routine clinical laboratory have been associated with measurands with a well-defined chemical structure and relative mass. This allows for the measurement procedures to be fully standardised using chemically pure components. The development of higher molecular mass LC-MSMS is likely to see the introduction of methods for proteins and other larger compounds of clinical interest. This will provide an additional challenge as many of these measurands do not have a clearly defined structure. Nevertheless, harmonisation of these analytes can be achieved and should be considered for harmonisation from the outset.

Conclusion
In conclusion, the profession has a unique and significant opportunity to bring clinical MS-based assays into agreement. Harmonisation of assays will ultimately provide the same result and interpretation for a given patient's sample, irrespective of the laboratory which produced the result. To achieve this goal, we need to continue to work together to reach agreement for the best practice LC-MSMS methods for use in routine clinical measurement.

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References

1. European Standards. In vitro diagnostic medical devices. Directive 98/79/EC. 1998. http://ec.europa.eu/enterprise/policies/european-standards/harmonised-standards/iv-diagnostic-medical-devices/ (Accessed 7 March 2012).
2. Biggs R, Denson KWE. Standardization of the one-stage prothrombin time for the control of anticoagulation therapy. Br Med J 1967;1:84-8.
3. WHO Expert Committee on Biological Standardization. 28th Report. World Health Organization Technical Report Series 610. Geneva: WHO; 1977. pp 14-15,185-205. http://whqlibdoc.who.int/trs/WHO_TRS_610.pdf (Accessed 9 July 2012).
4. WHO Expert committee on biological standardisation. 33rd report, WHO technical report series 687. 1983: pages 81-105. http://whqlibdoc.who.int/trs/WHO_TRS_687.pdf (Accessed 9 July 2012)
5. Poller L. International Normalized Ratios (INR): the first 20 years. J Thromb Haemost 2004;2:849-60.
6. Armbruster D, Miller RR. The Joint Committee for Traceability in Laboratory Medicine (JCTLM): a global approach to promote the standardisation of clinical laboratory test results. Clin Biochem Rev 2007;28:105-13.
7. The Nobel Prize in Physics 1906. http://www.nobelprize.org/nobel_prizes/physics/laureates/1906 (Accessed 9 March 2012).
8. Gohlke RS, McLafferty FW. Early gas chromatography/mass spectrometry. J Am Soc Mass Spectrom 1993;4:367-71.
9. Krone N, Hughes BA, Lavery GG, Stewart PM, Arlt W, Shackleton CHL. Gas chromatography/mass spectrometry (GC/MS) remains a pre-eminent discovery tool in clinical steroid investigations even in the era of fast liquid chromatography tandem mass spectrometry (LC/MS/MS). J Steroid Biochem Mol Biol 2010;121:496-504.
10. Joint Committee for Traceability in Laboratory Medicine (JCTLM) database of higher-order reference materials, measurement methods/procedures and services. http://www.bipm.org/jctlm/home.do (Accessed 2 July 2012).
11. The Nobel Prize in Chemistry 2002. http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2002/ (Accessed 9 March 2012).
12. International Standards Organization. Medical laboratories – Particular requirements for quality and competence. ISO 15189:2007. http://www.iso.org/iso/catalogue_detail?csnumber=42641 (Accessed 12 June 2012).
13. RCPA QAP Chemical Pathology Programs. Urine Chemistry and Biogenic Amines Program Cycle 55, 2012. http://www.rcpaqap.com.au/chempath (Accessed 12 March 2012)
14. United Kingdom National External Quality Assessment Service. UKNEQAS Steroid Hormone Program; 2012: Distribution 382. http://www.birminghamquality.org.uk/ (Accessed 12 March 2012).
15. Maynard S. Immunosuppressants: An update on therapeutic drug monitoring. Clin Lab News 2011; 37:8-10.
16. Greaves RF, Donath SM, Hunt RW. Hormone levels in extreme prematurity: Establishment of gestational appropriate reference intervals for the first six weeks of life. Clin Biochem 2011;44:531.
17. Shah I, James R, Barker J, Petroczi A, Naughton DP. Misleading measures in vitamin D analysis: a novel LC-MS/MS assay to account for epimers and isobars. Nutr J 2011;10:46.
18. Rauh M. Steroid measurement with LC-MS/MS. Application examples in pediatrics. J Steroid Biochem Mol Biol 2010;121:520-7.
19. Greaves R, Kanumakala S, Read A, Zacharin M. Genital abnormalities mimicking congenital adrenal hyperplasia in premature infants. J Paediatr Child Health 2004;40:233-6.
20. Pitt JJ. Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. Clin Biochem Rev 2009;30:19-34.
21. Annesley TM. Ion suppression in mass spectrometry. Clin Chem 2003;49:1041-4.
22. Miller WG, Myers GL, Rej R. Why commutability matters. Clin Chem 2006;52:553-4.
23. Siekmann L. Requirements for reference (calibration) laboratories in laboratory medicine. Clin Biochem Rev 2007;28:149-54.
24. Levine DM, Maine GT, Armbruster DA, Mussell C, Buchholz C, O'Connor G, et al. The need for standardization of tacrolimus assays. Clin Chem 2011;57:1739-47.
25. Armbruster DA, Pry T. Limit of blank, limit of detection and limit of quantitation. Clin Biochem Rev 2008;29:S49-S52.
26. Burns DT, Danzer K, Townshend A. Use of the terms "recovery" and "apparent recovery" in analytical procedures (IUPAC Recommendations 2002). Pure Appl Chem 2002;74:2201-5.
27. Clinical and Laboratory Standards Institute. Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline. CLSI Document C50-A. Wayne PA, USA: CLSI; 2007.
28. Westgard, JO, Quam E, Barry P, Ehrmeyer SL. Basic Method Validation: Training in Analytical Quality

Management for Healthcare Labor. 1st Edition. Madison, WI, USA: Westgard Quality Corporation; 1999. 250 pp.
29. Honour JW. Development and validation of a quantitative assay based on tandem mass spectrometry. Ann Clin Biochem 2011;48:97-111.
30. Stöckl D, Sluss PM, Thienpont LM. Specifications for trueness and precision of a reference measurement system for serum/plasma 25-hydroxyvitamin D analysis. Clin Chim Acta 2009;408:8-13.
31. Jones G, Barker A, Tate J, Lim C-F, Robertson K. The case for common reference intervals. Clin Biochem Rev 2004;25:99-104.
32. Clinical and Laboratory Standards Institute. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition. CLSI Document C28-A3c. Wayne PA, USA: CLSI; 2010.
33. Asian Pacific Conference of Chromatography and Mass Spectrometry: New Horizons in Clinical Chemistry. Hong Kong SAR, 14–16 January 2010. http://www.medicine.org.hk/hkscc/communication/2010/Mass%20Spectrometry.pdf (Accessed 12 March 2012).
34. Asian and Pacific Federation of Clinical Biochemists (APFCB) Mass Spectrometry Harmonisation Working Group. Minutes of meeting held at Hong Kong University, Hong Kong SAR, China. 6 January 2010.
35. White GH. Metrological traceability in clinical biochemistry. Ann Clin Biochem 2011;48:393-409.
36. Centers for Disease Control and Prevention. Inventory of External QA Programs for Clinical (Medical) Laboratories. http://wwwn.cdc.gov/mlp/EQA_programs.aspx (Accessed 2 April 2012).
37. Westgard JO. Basic Method Validation: Training in Analytical Quality Management for Healthcare Laboratories. 3rd Edition. Madison, WI, USA: Westgard QC Inc; 2008. 320 pp.
38. Centers for Disease Control. Laboratory Medicine Best Practices: Developing an Evidence-Based Review and Evaluation Process. Final Technical Report 2007. http://wwwn.cdc.gov/dls/bestpractices/ (Accessed 11 June 2012).
39. Joint Committee for Guides in Metrology. International vocabulary of metrology – Basic and general concepts and associated terms (VIM). Third edition; 2012. http://www.bipm.org/utils/common/documents/jcgm/JCGM_200_2012.pdf (Accessed 7 March 2012).
40. The International System of Units (SI). http://www.bipm.org/en/si/ (Accessed 7 March 2012).
41. Greaves RF. Vitamin A: Serum Vitamin A Analysis. In: Food and Nutritional Components in Focus: Chemistry, Analysis, Function and Effects. Preedy VR, editor. 1st Ed. Cambridge, UK: The Royal Society of Chemistry; 2012:162-83.
42. Joint Committee for Traceability in Laboratory Medicine. 2002. Appendix III, The JCTLM Framework: A Framework for the international recognition of available higher-order reference materials, available higher-order reference measurement procedures and reference measurement laboratories for laboratory medicine. http://www.bipm.org/utils/en/pdf/jctlm_framework.pdf (Accessed 7 June 2012).
43. Fasce CF Jr, Rej R, Copeland WH, Vanderlinde RE. A discussion of enzyme reference materials: applications and specifications. Clin Chem 1973;19:5-9.
44. Clinical and Laboratory Standards Institute. Characterization and Qualification of Commutable Reference Materials for Laboratory Medicine; Approved Guideline. CLSI document C53-A. Wayne PA, USA: CLSI; 2010.
45. Fraser CG. Quality Specifications. In: Biological Variation: From Principles to Practice. Washington, DC, USA: AACC Press; 2001. pp29-66.
46. White GH, Farrance I. Uncertainty of measurement in quantitative medical testing: a laboratory implementation guide. Clin Biochem Rev 2004;25:S1-24.
47. Ricós C, Alvarez V, Cava F, García-Lario JV, Hernández A, Jiménez CV, et al. Current databases on biological variation: pros, cons and progress. Scand J Clin Lab Invest 1999;59:491-500. http://www.westgard.com/biodatabase1.htm (Accessed 4 March 2012).