Clinica Chimica Acta 546 (2023) 117391

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

LC-MS/MS in clinical chemistry: Did it live up to its promise? Consideration from the Dutch EQAS organisation

E.G.W.M. Lentjes [a,*], H.N. Bui [b], L.R. Ruhaak [c], I.P. Kema [d], K.L.M. Coene [e], J.M.W. van den Ouweland [f]

[a] Central Diagnostic Laboratory, University Medical Center Utrecht, the Netherlands
[b] Clinical Chemistry, Reinier de Graaf Groep Diagnostisch Centrum SSDZ, the Netherlands
[c] Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, the Netherlands
[d] Department of Laboratory Medicine, University Medical Center Groningen, the Netherlands
[e] Laboratory of Clinical Chemistry and Hematology, Máxima Medical Centre, Veldhoven, the Netherlands
[f] Department of Clinical Chemistry, Canisius-Wilhelmina Hospital, Nijmegen, the Netherlands

ARTICLE INFO

Keywords:
LC-MS/MS
Interlaboratory imprecision
EQAS
Steroids

ABSTRACT

Background: Over the past decade the use of LC-MS/MS has increased significantly in the hospital laboratories. Clinical laboratories have switched from immunoassays to LC-MS/MS methods due to the promise of improvements in sensitivity and specificity, better standardization with often non-commutable international standards, and better between-laboratory comparison. However, it remains unclear whether routine performance of the LC-MS/MS methods have met these expectations.

Method: This study examined the EQAS results, from the Dutch SKML, of serum cortisol, testosterone, 25OH-vitaminD and cortisol in urine and saliva over 9 surveys (2020 to first half of 2021).

Results: The study found a significant increase in the number of compounds and in the number of results measured in the different matrices, with LC-MS/MS over a period of eleven years. In 2021, approximately 4000 LC-MS/MS results were submitted (serum: urine: saliva = 58:31:11%) compared to only 34 in 2010.

When compared to the individual immunoassays, the LC-MS/MS based methods for serum cortisol, testosterone and 25OH-vitaminD showed comparable but also higher between-laboratory CVs in different samples of the surveys. For cortisol, testosterone and 25OH-vitaminD the median CV was 6.8%, 6.1% and 4.7% respectively for the LC-MS/MS compared to 3.9–8.0%,4.5–6.7%, and 7.5–18.3% for immunoassays. However, the bias and imprecision of the LC-MS/MS was better than that of the immunoassays.

Conclusion: Despite the expectation that LC-MS/MS methods would result in smaller between-laboratory differences, as they are relatively matrix independent and better to standardize, the results of the SKML round robins do not reflect this for some analytes and may be in part explained by the fact that in most cases laboratory developed tests were used.

1. Introduction

For the past fifty years hormone analysis has been carried out using immunoassays (IAs). While these assays have many advantages such as sensitivity, relatively speed of analyses, automation, wide applicability, their effective use require not only technical skills but also knowledge of potential pitfalls. One major issue with immunoassays is interference from cross-reacting substances, especially at low analyte concentrations, as demonstrated for example for testosterone in neonates [1], or for 25hydroxyvitaminD (25OH-VitD) [2]. Binding proteins can also cause interference, as seen with cortisol [3]. With the introduction of mass spectrometry in the clinical laboratories a new technique came available to overcome these problems [4]. The benefits that LC-MS/MS methods offer over immunoassays for small molecular compounds, such as

Abbreviations: IS, internal standard; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LDT, laboratory developed test; SKML, Foundation for Quality Control of Medical Laboratories; TEa, total allowable error; 25OH-VitD, 25hydroxyvitaminD.

* Corresponding author at: University Medical Center Utrecht, the Netherlands/Central Diagnostic Laboratory G03.555 / Heidelberglaan 100, 3584CX Utrecht, the Netherlands.

E-mail address: elentjes@umcutrecht.nl (E.G.W.M. Lentjes).

https://doi.org/10.1016/j.cca.2023.117391
Received 29 December 2022; Received in revised form 9 May 2023; Accepted 10 May 2023
Available online 15 May 2023

steroids and vitamins, were addressed a decade ago [5,6] and include the use of stable isotope labeled internal standards (IS), sensitivity and specificity. It was not until 2010 when the first LC-MS/MS determined results (for 25OH-VitD) were submitted to the Dutch external quality assurance program (SKML, Foundation for Quality Control of Medical Laboratories). In the years that followed, an almost exponential rise in LC-MS/MS submitted results was observed.

LC-MS/MS has become a routine diagnostic tool in clinical laboratories and has undergone several innovations over the past 10–20 years allowing for its integration into daily diagnostic practice. This innovations include automated pre-analytical procedures, faster analysis, increased sensitivity and specificity of the instruments, the possibility of profiling, an increasing availability of ready-to-use kits, and improved software making it easier to use. Additionally, there has been an exponential increase in the number of publications on LC-MS/MS analyses on a wide range of analytes.

Mass-spectrometry is often used for the development of a 'gold standard' method not only for the measurement of small molecular analytes such as steroids, but also for peptides, proteins and metabolites. Many LC-MS/MS based methods can be found in the database of The Joint Committee for Traceability in Laboratory Medicine [7]. Given the superior performance of LC-MS/MS, the Endocrine Society recommended in a Position Statement that LC-MS/MS should be used for the measurement of testosterone and free testosterone [8]. Furthermore, there is a trend in guidelines recommending LC-MS/MS, for example for measurement of steroids in congenital adrenal hyperplasia [9] or in polycystic ovary syndrome [10].

Most clinical laboratories use LC-MS/MS to measure low molecular weight compounds, often in a multiplex panel. However, for some methods, IAs are the preferred or even the only option due to the urgent need for test results in acute patient care setting.

There are high expectations regarding the performance of LC-MS/MS, but the question remains whether these expectations have been met. The study aims is to evaluate the between- and intra-laboratory performance of LC-MS/MS results compared to immunoassay methods for some analytes, by reviewing 9 surveys of EQAS data (between 2020 and 2022).

2. Materials and methods

2.1. EQA round robins

The Dutch external quality organization (SKML) organizes 6 round robins annually for hormones and vitamins in blood, urine, and saliva. Each round robin includes two specimens of frozen human serum, urine, or saliva, without stabilizers, each containing different concentrations of hormones and/or vitamins. The basic material used is serum from healthy blood bank donors, or saliva and urine from healthy individuals. In some rounds serum from only men, or only women either below 40 years or above 50 years of age, is used to obtain high or low concentrations of testosterone or estradiol. About half of the samples are spiked with the appropriate steroids. Every year, twelve samples are sent on dry ice to the participants. The participants analyze the appropriate sample each month and report the results online to the SKML. The SKML sends a report after the completion of a set of two samples, resulting in six reports being sent to the participants.

Currently, target values are set per sample for testosterone, cortisol, 25OH-VitD. The concentrations in the EQAS samples of steroids, that were used for the Figs. 2 to 4, ranged for cortisol (serum) from 222 to 1085 nmol/L, for testosterone from 0.97 to 43 nmol/L, for 25OH-VitD from 55 to 133 nmol/L, for cortisol (urine) from 89 to 646 nmol/L and for cortisol (saliva) from 0.6 to 47 nmol/L. About half of the samples are native samples the other half are spiked with the appropriate steroid.

Cortisol target values were established based on the results from an LC-MS/MS method that participates in an independent international EQA program. The samples were determined based on a GC-IDMS reference method and the IFCC cortisol reference serum panel. Traceability was ensured by using NIST 921 certified standard, with an accuracy matrix check using ERM-DA192 and ERM-DA193, which are IRMM certified cortisol samples in lyophilized human serum.

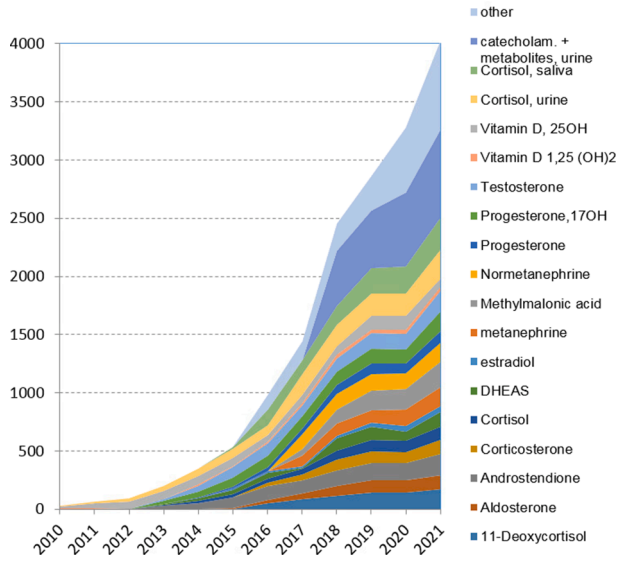

Fig. 1. Number of results per year for hormones, vitamins and metabolites in EQAS samples obtained by LC-MS/MS measurements. All parameters are in serum, unless indicated otherwise.

For testosterone a CDC-certified testosterone LC-MS/MS method was used for the assignment of targets [11]. For 25OH-VitD the median value of four LC-MS/MS laboratories was used. These laboratories participated in an interlaboratory survey, using samples measured with a reference measurement procedure [2]. For cortisol in urine and saliva, the all laboratory trimmed mean (e.g. excluding outliers) was used as the target value for each sample.

2.2. Statistical analysis

Statistical analysis was performed using IBM SPSS statistical software for Windows version 27.0 (IBM Corp., Armonk, N.Y., USA). The data presented in this study was collected from the EQAS 2010–2021 schemes of hormones of the Dutch SKML and all data were anonymized. Method comparison was possible for cortisol, testosterone and 25OH-VitD in serum, as results from both LC-MS/MS and several IA methods were available. For cortisol in urine and saliva both LC-MS/MS and, when available, IA methods were used. The within-method coefficient of variation (CV) was calculated for each method per sample. At least 4 laboratories using the same method were required to provide their results per sample, measured in singular as for patient samples. The CV's of 18 samples, covering 9 rounds, from 2020 and 2021 (round 1–3) were grouped. For cortisol and testosterone (for which log-transformed data were used) average CVs per sample were statistically compared only between the LC-MS/MS method and the IAs using the Welch's one-way ANOVA, because of unequal variances, and post hoc Tamhane multiple comparison test (adjusted P values < 0.05). For 25OH-VitD the non-parametric Kruskal-Wallis H test was used.

For rounds of 2020 and 2021 (round 1–3) the mean percentage of bias and the standard deviation of these bias values were calculated for four steroids. The mean within-laboratory bias was determined by calculating the average bias per round, compared to the target value for cortisol, testosterone and 25OH-VitD, or compared to the median value of the results per sample for cortisol in urine.

Sigma metrics can be used as a tool to assess the performance of an analytical assay on the Six Sigma scale based on the total allowable error (TEa) and the bias. The ideal goal is to achieve Six Sigma, meaning that

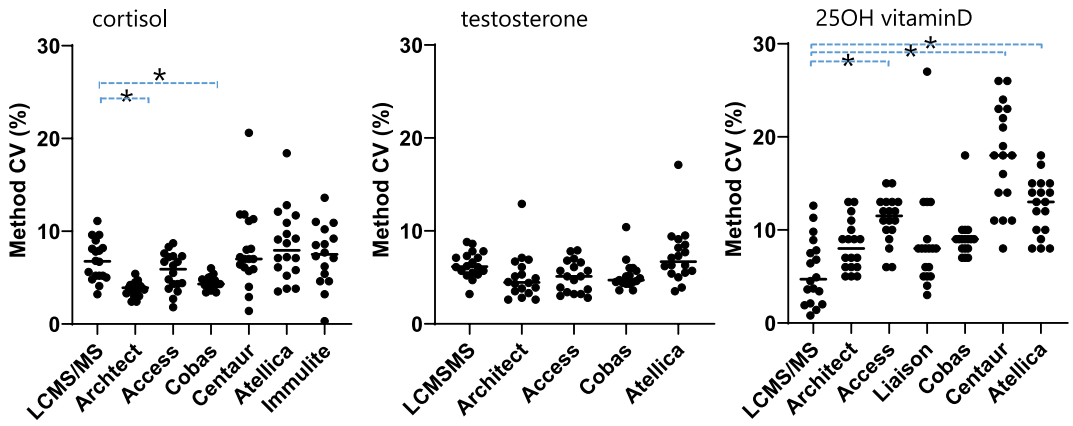

Fig. 2. Average between-laboratory CV per sample for serum cortisol, testosterone and vit-D, as grouped by method, for 18 samples in the Dutch EQAS. Results from 2020 (round robin 1 to 6) and 2021(1 to 3). The median value of each method is given (horizontal line). Only statistical significant difference (adjusted $p<0.05$) between LC-MS/MS and an immunoassay is given by an asterisk.

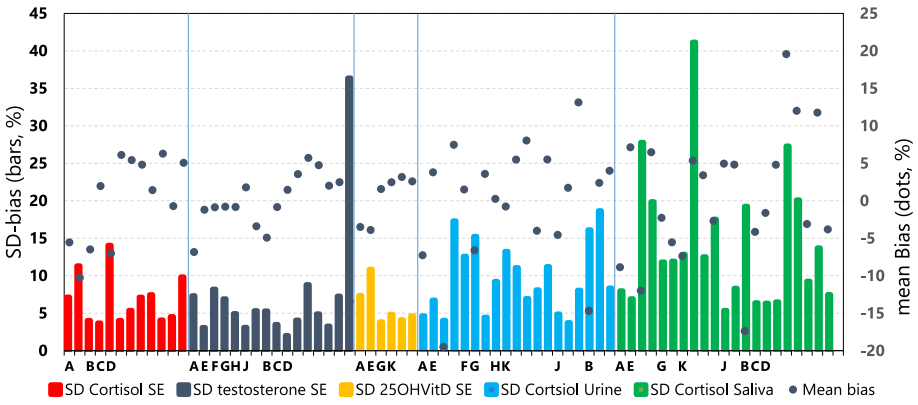

Fig. 3. LC-MS/MS performance over 9 rounds (18 samples) of individual laboratories for five analytes. Same letter on X-axis corresponds to same laboratory. The average within-laboratory bias (right Y-axis; dots) and average imprecision (SD) of the bias (left Y-axis; bars) is given for the year 2020 to the first half of 2021.

six standard deviations of an analytical procedure can fit within the defined tolerance limits. Three-Sigma (3σ) is commonly accepted as the minimum quality (marginal performance) for a procedure while 2σ indicates poor performance [12]. A figure can be constructed with the observed imprecision (X-axis) and the observed inaccuracy (Y-axis). Lines, representing 2σ and 3σ quality are drawn from + TEa and -TEa (Y-intercept) to TEa/2 and TEa/3 (X-intercept) for the 2σ, and 3σ lines.

The Sigma quality of an analytical method can be calculated as follows [13]:

Sigma = (%TEa - |%bias|) / %CV and TEa = Bias + 1.65*0.5*CVi.

The bias is the observed systematic error, which is calculated as $0.25*(CVi^2 + CVg^2)^{½}$, were CVi and CVg are respectively the intra-individual and interindividual CV for the analytical method.

The mean bias per method was calculated by comparing it to the target value of the EQA sample. The values of CVi and CVg for testosterone, cortisol and 25OH-VitD were obtained from the EFLM database, and the calculated TEa values were 16.5%, 26%, and 12.4% respectively. [14]. However for urinary cortisol only one study was included in the EFLM database and for salivary cortisol two studies were available, but they had significantly different VCi and VCg values [15]. For urinary cortisol a TEa of 65% can be calculated while for salivary cortisol we used the higher CVi and CVg and calculated a TEa of approximately 40. %.

3. Results

The number of LC-MS/MS results increased from 34 in 2010, 333 in 2015 with ratios of serum, urine, and saliva samples of 58%, 37% and 5 %, respectively. This number has increased to over 4000 in 2021 with serum, urine, and saliva ratios of 58%, 31%, and 11% respectively. Fig. 1 illustrates this increase of SKML results obtained by LC-MS/MS methods over time. Results for cortisol in urine and saliva, serum 17-OH progesterone and androstenedione are now almost exclusively reported using LC-MS/MS. In a recent survey (round robin 2022–1) 27% of all results in the steroid and vitamin selection of methods were determined with LC-MS/MS.

In the Dutch EQAS reports it was striking that on average the between-laboratory CVs of LC-MS/MS methods were comparable to those of IAs, but in some of individual samples, were sometimes higher compared to those of IAs. Fig. 2 provides an overview of the variability per method for serum cortisol, testosterone and 25OH-VitD. Each dot represents an average between-laboratory CV for each sample, for the rounds 2020(1 to 6) and 2021 (1 to 3). The number of the laboratories for each method is given in Table 1. A statistical significant difference between the LC-MS/MS and the different IAs is indicated by an asterisk. For cortisol the between-laboratory CV was highest for the LC-MS/MS method in 3 out of 18 samples, and for testosterone it was highest in 1 out of 18 samples. However, for 25OH-VitD, the LC-MS/MS method had the lowest CV in 9 out of 18 samples. For cortisol the median CV is 6.8% for the LC-MS/MS compared to 3.9–8.0% for the median CV's of the IAs. For testosterone it is 6.1% compared to 4.5–6.7% respectively and for 25OH-VitD it is 4.7% compared to 7.5–18.3% respectively. For 25OH-VitD, LC-MS/MS method has the lowest median CV.

In Fig. 3, the mean bias and standard deviation (SD) of the bias

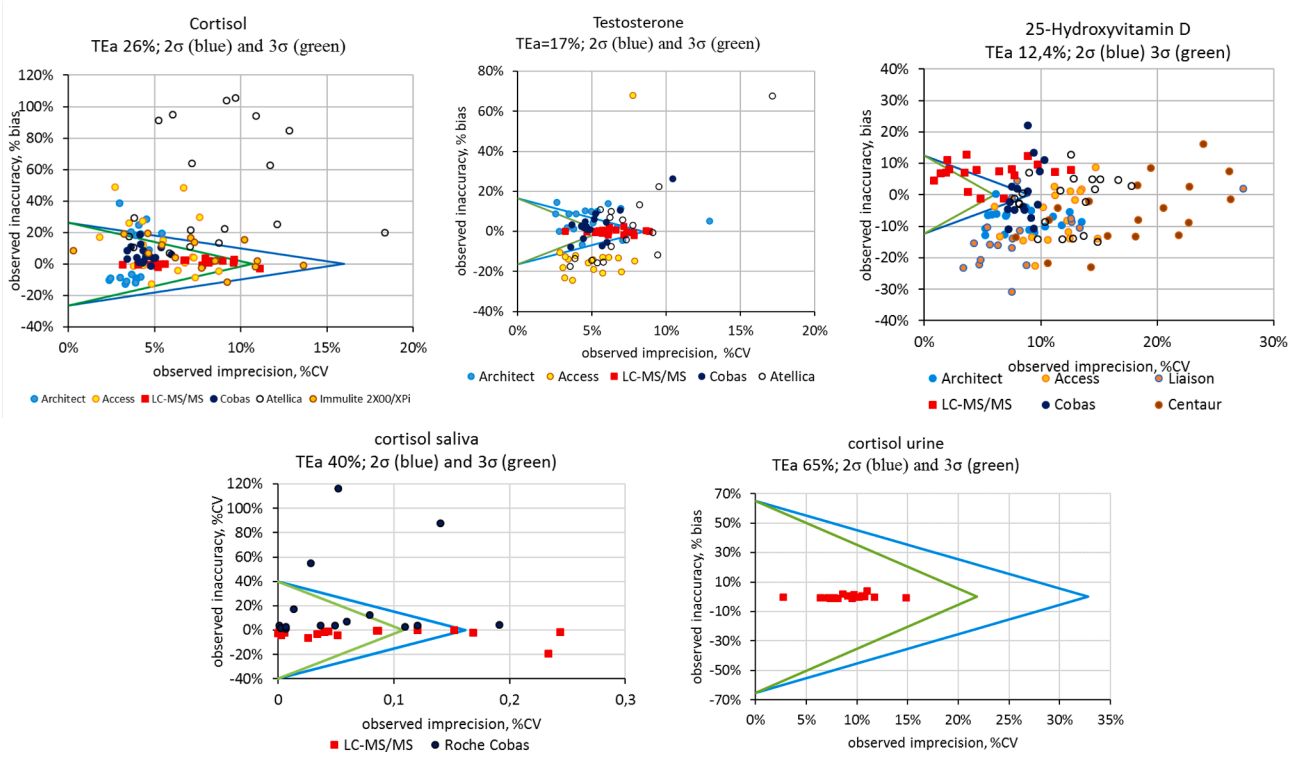

Fig. 4. Sigma Proficiency Assessment Chart for serum cortisol, testosterone, 25-hydroxyvitaminD and for cortisol in saliva and in urine with TEa=32%, 17%, 18%, 33% and 43% respectively. Y-axis represent the observed inaccuracy (% bias); X-axis the observed imprecision (%CV). Diagonal lines represent 2sigma (blue) and 3sigma (green) limits. Each point represent the observed percentage bias (Y-axis) and the observed percentage imprecision (X-axis) for a specific sample-method combination from results of 2020 (round robin 1 to 6) and 2021(1 to 3).

Table 1
Number of laboratories for each analyte, per method and per sample.

				cortisol	
	cortisol	**testosterone**	**25OH-vitaminD**	**Saliva**	**Urine**
LC-MS/MS	8–10	11–15	5–6	17–24	16–22
Architect	6–8	5–7	5–7	NA	NA
Access	6–9	5–9	8–11	NA	NA
Liaison	NA	NA	5–7	NA	NA
Cobas	46–50	37–40	50–58	6–9	NA
Centaur	5–6	NA	5–6	NA	NA
Atellica	7–9	5–8	6–11	NA	NA
Immulite	5–7	NA	NA	NA	NA

NA = not available.

resulting from 2020 (round 1 to 6) and 2021 (round 1to 3) of individual laboratories only using LC-MS/MS is shown. In addition to the data from serum cortisol, testosterone and 25OH-VitD, urinary and salivary cortisol, for which only LC-MS/MS data were available, are also presented. The average bias (range) for serum cortisol is −0.24% (-10.3 to + 6.3%), for testosterone −0.71% (-13.4 to + 5.8%), for 25OH-VitD + 0.55% (range −3.5 to + 3.2%) and for cortisol in urine −0.10% (-14.7 to + 11.5%) and for cortisol in saliva: 0.54 (-17.3 to + 19.6%). Large differences between laboratories are observed, and some laboratories show 3 to 4 times higher SD(bias) than others. During the investigated period, some laboratories sent results for more analytes (same letter on x-axis). Laboratory A performed for all five analytes with an SD-bias and mean bias below 10%, and laboratory C, E, J for three analytes, while the others performed more variable, mostly with a higher SD-bias. The high bar (bias) in the testosterone part of the figure for one laboratory is due to abnormal results in various rounds. Overall, 10 of the 74 mean bias results (dots in Fig. 3), produced by 51 laboratories, exceed 10% for one or more of the analytes, and 30 results have a bias exceeding 5%.

The results of the average imprecision and bias per method for IAs and LC-MS/MS for serum cortisol, testosterone, 25OH-VitD, and for urine and saliva cortisol (where only LC-MS/MS methods were reported) are presented in a Sigma metrics plot (Fig. 4). A sigma proficiency assessment chart was constructed from survey data of 18 EQA samples (2020 to 2021 round 1–3) according to Westgard [12]. It is common practice to use 3-sigma as the minimum quality requirement. For serum testosterone, cortisol, and 25OH-VitD, the observed inaccuracy (%bias) per method for individual EQA samples for IAs often exceeds the 2 sigma lines (blue lines), and for 25OH-VitD IAs, it is also due to higher imprecision. The LC-MS/MS methods show imprecision and bias results that exceed the 3 sigma lines: for serum cortisol, testosterone, 25OH-VitD, cortisol in urine and cortisol in saliva, it is 1, 13, 12, 0, and 5 data points, respectively, and for the 2 sigma lines it is 0, 2, 10, 0, and 3 data points respectively. For testosterone the Access IA method showed a median bias of −14% and only one of the data points lie within the 3 sigma lines. It is remarkable that the LC-MS/MS methods for 25OH-VitD show an average bias of + 6.8% (-1 to + 13%).

4. Discussion

The introduction of the LC-MS/MS in the diagnostic laboratories have many advantages, as previously discussed. A major advantage in pediatric endocrinology is the profiling capability of a steroid panel, especially when limited sample volumes are available. In comparison to IAs, LC-MS/MS offers relatively low reagent cost per sample. However, the high costs of purchasing LC-MS/MS equipment and the need for well-trained technicians can be significant obstacles to implementing the LC-MS/MS in many diagnostic laboratories [6]. Additionally, LC-MS/MS analyses are usually performed in batches, making it difficult to obtain results on the same day, let alone within 1 to 2 h as is possible with automated IA platforms.

LC-MS/MS is commonly used for small molecules in blood, urine,

and saliva and its value in routine diagnostics is evident in the exponential increase in reported results in the Dutch EQAS for endocrinology. Moreover, an increase in the number of routinely measured analytes with LC-MS/MS over the years has been observed.

Results from the Dutch EQAS indicate that for certain analytes, the LC-MS/MS within-method CV is not consistently better than that of the IAs. For a few serum samples the LC-MS/MS within-method CV for cortisol and testosterone were occasionally higher than that of some IAs. For 25OH-VitD, however, the median method-CV is lower for LC-MS/MS than for the IAs, although there is overlap in the range of individual CV's per sample with the IAs. This is also reflected in Fig. 3, where a large variation in the SD-bias is seen, especially for cortisol in urine and saliva, among laboratories that use LC-MS/MS. During the investigated period, certain laboratories (identified by the same letter on the X-axis), submitted results for more analytes, with low SD-bias and mean bias, while others exhibited greater variability. Although based on small numbers, it might indicate that some laboratories have more reliable and stable methods.

One of the main advantages for LC-MS/MS analysis is better standardization due to its relative matrix independence and better selectivity than IAs, which rely on the antibodies used. Despite this, as shown in Figs. 2 and 4, the observed average imprecision of the LC-MS/MS methods for serum cortisol and testosterone is no better than that of the different IAs. For testosterone in Fig. 4, the negative bias of the Access IA for 17 out of 18 samples, suggests a standardization issue The negative bias was comparable to the average 9% lower results in a recent comparison of the Access analyzer to an LC-MS/MS method [16].

For 25OH-VitD, the imprecision is, on average, better than that of the IAs. However, the bias of the LC-MS/MS methods, as seen in Fig. 4 is superior to those of the IAs, with a small range of bias, near zero bias, for the 18 EQA samples compared to a much broader range for the IAs. The average bias of + 6.8% for the LC-MS/MS method might be explained by interference of some methods by the 3-epi-25OH-VitD [17].

Several factors may contribute to the relatively high imprecision, including the fact that many LC-MS/MS methods used in diagnostic laboratories are laboratory-developed tests (LDTs), often due to the absence of commercial kits or because the need for a specific combination of analytes. The LDTs in use in the different laboratories differ in several steps of the complete procedure, such as sample handling, chromatographic conditions, LC-MS/MS settings, use of different standards and internal standards, no uniformity in the system suitability checks, difference in skills of technicians and differences in the criteria used for the evaluation of the assays.

4.1. How to improve the LC-MS/MS performance?

How can the performance of LC-MS/MS methods be improved? The use of common calibrators for LDT LC-MS/MS methods has been shown to lead to an improvement, as demonstrated for the 25OH-VitD assay method [18], and for tacrolimus [19]. However, Owen et al demonstrated in an experiment amongst fifteen LC-MS/MS LDT users for testosterone in the UKNEQAS that the use of common serum based calibrators did not improve between-laboratory CV [20]. Therefore, the use of a common calibrator might be part of the solution, but other factors in the preparation and analysis of the samples and calibrators are also crucial to improve LC-MS/MS performance. Even the choice of an IS can affect the LC-MS/MS results: Owen and Keevil demonstrated that the use of D5 and 3C13 IS for testosterone gave lower results compared to the use of a D2 IS [21]. Moreover, in hospital laboratories working with highly complex blood, urine, and saliva samples with potential drug interference and variable protein and electrolyte composition, as well as different MS instruments, a thorough investigation of matrix effects and transition selectivity should be an essential part of the method validation.

Before the introduction of a method in routine clinical diagnostics, it is important to subject the method to careful and structured validation. Guideline C62-A of the Clinical and Laboratory Standard Institute (CLSI), provides guidance for, among other things, LC-MS/MS method development [2223]. Vogeser et al have also published a set of 35 fundamental and 15 variable method characteristics as a tool to stablish a universal description of any LC-MS/MS method, making it easier to implement a method, developed in one laboratory in other laboratories with different equipment and conditions [24]. Recently, there has been useful adaptations to the CLSI 62-A guideline for LC-MS/MS method validation when negative (lacking the substance of interest) samples are not available [2]. When laboratories take the time and have the discipline to describe their LC-MS/MS methods in detail as suggested by Vogeser and Stone and follow the CLSI C62-A guideline for validation, this is likely to improve the quality of LC-MS/MS assays. Additionally, laboratories in the European Union are required to comply with the new EU IVDR regulations (2017/746), which state that LDTs may only be used when there is no equivalent commercial device available on the market with an appropriate level of analytical and clinical performance [25], which further emphasizes the need for laboratories to improve their LDTs.

5. Conclusion

LC-MS/MS has now established a permanent place in clinical diagnostic laboratories. While the overall bias is better compared to the IAs, for some analytes, there is still room for improvement in terms of between laboratory variation. Several factors are likely to contribute to this variation. Hopefully, this variation can be minimized through standardization and a rigorous implementation of validation protocols, enabling LC-MS/MS to fully deliver on its promises.

CRediT authorship contribution statement

E.G.W.M. Lentjes: Conceptualization, Writing – original draft, Formal analysis, Investigation, Visualization. **H.N. Bui:** Conceptualization, Writing – review & editing. **L.R. Ruhaak:** Conceptualization, Writing – review & editing. **I.P. Kema:** Conceptualization, Writing – review & editing. **K.L.M. Coene:** Conceptualization, Writing – review & editing. **J.M.W. van den Ouweland:** Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sector

References

[1] H.M. Hamer, M.J.J. Finken, A.E. Van Herwaarden, T. Du Toit, A.C. Swart, A. C. Heijboer, Falsely elevated plasma testosterone concentrations in neonates: Importance of LC-MS/MS measurements, Clin. Chem. Lab. Med. 56 (2018) e141–e143, https://doi.org/10.1515/cclm-2017-1028.

[2] N.F. Dirks, H.W. Vesper, A.E. van Herwaarden, J.M.W. van den Ouweland, I. P. Kema, J.G. Krabbe, A.C. Heijboer, Various calibration procedures result in

optimal standardization of routinely used 25(OH)D ID-LC-MS/MS methods, Clin. Chim. Acta 462 (2016) 49–54, https://doi.org/10.1016/j.cca.2016.08.016.
[3] A. Gant Kanegusuku, K.A. Araque, H. Nguyen, B. Wei, S. Hosseini, S.J. Soldin, The effect of specific binding proteins on immunoassay measurements of total and free thyroid hormones and cortisol, Ther. Adv. Endocrinol. Metab. 12 (2021) 1–9, https://doi.org/10.1177/2042018821989240.
[4] A.N. Hoofnagle, M.H. Wener, The fundamental flaws of immunoassays and potential solutions using tandem mass spectrometry, J. Immunol. Methods. 347 (2009) 3–11, https://doi.org/10.1016/j.jim.2009.06.003.
[5] V.M. Carvalho, The coming of age of liquid chromatography coupled to tandem mass spectrometry in the endocrinology laboratory, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 883–884 (2012) 50–58, https://doi.org/10.1016/j.jchromb.2011.08.027.
[6] J.M.W. Van den Ouweland, I.P. Kema, The role of liquid chromatography-tandem mass spectrometry in the clinical laboratory, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 883–884 (2012) 18–32, https://doi.org/10.1016/j.jchromb.2011.11.044.
[7] JCTLM database, The Joint Committee for Traceability, (n.d.). https://www.bipm.org/jctlm/.
[8] W. Rosner, R.J. Auchus, R. Azziz, P.M. Sluss, H. Raff, Position statement: Utility, limitations, and pitfalls in measuring testosterone: An endocrine society position statement, J. Clin. Endocrinol. Metabol. 92 (2007) 405–413, https://doi.org/10.1210/jc.2006-1864.
[9] P.W. Speiser, W. Arlt, R.J. Auchus, L.S. Baskin, G.S. Conway, D.P. Merke, H.F. L. Meyer-Bahlburg, W.L. Miller, M.H. Murad, S.E. Oberfield, P.C. White, Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an endocrine society clinical practice guideline, J. Clin. Endocrinol. Metab. 103 (11) (2018) 4043–4088.
[10] H.J. Teede, M.L. Misso, M.F. Costello, A. Dokras, J. Laven, L. Moran, T. Piltonen, R. J. Norman, M. Andersen, R. Azziz, A. Balen, E. Baye, J. Boyle, L. Brennan, F. Broekmans, P. Dabadghao, L. Devoto, D. Dewailly, L. Downes, B. Fauser, S. Franks, R.M. Garad, M. Gibson-Helm, C. Harrison, R. Hart, R. Hawkes, A. Hirschberg, K. Hoeger, F. Hohmann, S. Hutchison, A. Joham, L. Johnson, C. Jordan, J. Kulkarni, R.S. Legro, R. Li, M. Lujan, J. Malhotra, D. Mansfield, K. Marsh, V. McAllister, E. Mocanu, B.W. Mol, E. Ng, S. Oberfield, S. Ottey, A. Peña, J. Qiao, L. Redman, R. Rodgers, L. Rombauts, D. Romualdi, D. Shah, J. Speight, P.M. Spritzer, E. Stener-Victorin, N. Stepto, J.S. Tapanainen, E. C. Tassone, S. Thangaratinam, M. Thondan, C.R. Tzeng, Z. van der Spuy, E. Vanky, M. Vogiatzi, A. Wan, C. Wijeyaratne, S. Witchel, J. Woolcock, B.O. Yildiz, Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome, Fertil Steril. 110 (2018) 364–379, https://doi.org/10.1016/j.fertnstert.2018.05.004.
[11] CDC, Clinical Standardization Programs, (n.d.). https://www.cdc.gov/labstandards/csp/hs_host_certification.html.
[12] J.O. Westgard, Sten A.;Bayat, Hassan; Westgard, Special issue : Analytical Sigma metrics: A review of Six Sigma implementation tools for medical laboratories, Biochem Med (Zagreb). 28 (2018) 1–9.
[13] J.O. Westgard, S.A. Westgard, Assessing quality on the Sigma scale from proficiency testing and external quality assessment surveys, Clin. Chem. Lab. Med. 53 (2015) 1531–1535, https://doi.org/10.1515/cclm-2014-1241.
[14] S.S. Aarsand AK, Fernandez-Calle P, Webster C, Coskun A, Gonzales-Lao E, Diaz-Garzon J, Jonker N, Minchinela J, Simon M, Braga F, Perich C, Boned B, Roraas T, Marques-Garcia F, Carobene A, Aslan B, Barlett WA, The EFLM Biological Variation Database. https://biologicalvariation.eu/, (2021).
[15] Å.M. Hansen, A.H. Garde, R. Persson, Sources of biological and methodological variation in salivary cortisol and their impact on measurement among healthy adults: A review, Scand. J. Clin. Lab. Invest. 68 (2008) 448–458, https://doi.org/10.1080/00365510701819127.
[16] R. Dittadi, M. Matteucci, E. Meneghetti, R. Ndreu, Reassessment of the Access Testosterone chemiluminescence assay and comparison with LC-MS method, J. Clin. Lab. Anal. 32 (3) (2018) e22286.
[17] J.M.W. van den Ouweland, A.M. Beijers, H. van Daal, Overestimation of 25-hydroxyvitamin D3 by increased ionisation efficiency of 3-epi-25-hydroxyvitamin D3 in LC-MS/MS methods not separating both metabolites as determined by an LC-MS/MS method for separate quantification of 25-hydroxyvitamin D3, 3-epi-25-h, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 967 (2014) 1950–11202, https://doi.org/10.1016/j.jchromb.2014.07.021.
[18] G.D. Carter, J.C. Jones, Use of a common standard improves the performance of liquid chromatography-tandem mass spectrometry methods for serum 25-hydroxyvitamin-D, Ann. Clin. Biochem. 46 (2009) 79–81, https://doi.org/10.1258/acb.2008.008135.
[19] T.M. Annesley, D.A. Mckeown, D.W. Holt, C. Mussell, E. Champarnaud, L. Harter, L.J. Calton, D.S. Mason, Standardization of LC-MS for therapeutic drug monitoring of tacrolimus, Clin. Chem. 59 (2013) 1630–1637, https://doi.org/10.1373/clinchem.2013.209114.
[20] L.J. Owen, P.R. MacDonald, B.G. Keevil, Is calibration the cause of variation in liquid chromatography tandem mass spectrometry testosterone measurement? Ann. Clin. Biochem. 50 (2013) 368–370, https://doi.org/10.1177/0004563212474563.
[21] L.J. Owen, B.G. Keevil, Testosterone measurement by liquid chromatography tandem mass spectrometry: The importance of internal standard choice, Ann. Clin. Biochem. 49 (2012) 600–602, https://doi.org/10.1258/acb.2012.012037.
[22] P.A. Wayne, Liquid chromatography-mass spectrometry methods; Approved guideline. Document C62-A. Clinical Laboratory Standards Institute., CLSI. (2014).
[23] K.L. Lynch, CLSI C62-A: A new standard for clinical mass spectrometry, Clin. Chem. 62 (2016) 24–29.
[24] M. Vogeser, J.A. Stone, A suggested standard for validation of LC-MS/MS based analytical series in diagnostic laboratories, Clin. Mass Spectrom. 16 (2020) 25–32, https://doi.org/10.1016/j.clinms.2020.02.002.
[25] IVDR 2017/746, Consolidated text: Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU, (2017). https://eur-lex.europa.eu/eli/reg/2017/746/2017-05-05.