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Interlaboratory comparison study of immunosuppressant analysis using a fully automated LC-MS/MS system

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Abstract

Objectives: All guidelines recommend LC-MS/MS as the analytical method of choice for the quantification of immunosuppressants in whole blood. Until now, the lack of harmonization of methods and the complexity of the analytical technique have prevented its widespread use in clinical laboratories. This can be seen in international proficiency schemes, where more than half of the participants used immunoassays. With the Cascadion SM Clinical analyzer (Thermo Fisher Scientific, Oy, Vantaa, FI) a fully automated LC-MS/MS system has been introduced, which enables the use of LC-MS/MS without being an expert in mass spectrometry.

Methods: To verify the interlaboratory comparison of the immunosuppressant assay on this type of instrument, three centers across Europe compared 1097 routine whole blood samples, each site sharing its own samples with the other two. In other experiments, the effects of freezing and thawing of whole blood samples was studied, and the use of secondary cups instead of primary tubes was assessed.

Results: In the Bland–Altman plot, the comparison of the results of tacrolimus in fresh and frozen samples had an average bias of only 0.36%. The respective data for the comparison between the primary and secondary tubes had an average bias of 1.14%. The correlation coefficients for patient samples with cyclosporine A (n=411), everolimus (n=139), sirolimus (n=114) and tacrolimus (n=433) were 0.993, 0.993, 0.993 and 0.990, respectively.

Conclusions: The outcome of this study demonstrates a new level of result harmonization for LC-MS/MS based immunosuppressant analysis with a commercially available fully automated platform for routine clinical application.

Keywords: automation; immunosuppressants; LC-MS/MS; therapeutic drug monitoring.

Introduction

Since the introduction of the first immunosuppressive drugs, there has been a need to optimize therapy and minimize side effects or rejection through the monitoring of drug levels in the bloodstream. The prevention of organ rejection, or even graft loss, remains the core objective of effective treatment.

The four most commonly monitored immunosuppressive drugs today are tacrolimus (TAC), cyclosporine A (CsA), sirolimus (SIR) and everolimus (EVE). There are consensus reports for optimal monitoring of all of these drugs requiring the analysis of whole blood [1–4]. In these consensus reports and other guidelines [5, 6], the use of a very specific and accurate analytical method is recommended which is now liquid chromatography mass spectrometry (LC-MS). The pre-requisite for success is a proper implementation of this technology as outlined by others [7]. For meaningful Therapeutic Drug Monitoring (TDM) timely delivery of results that facilitate fast and effective patient management is essential. The speed of results provision was until recently, a balance of the use of rapid but analytically flawed immunoassay (IA) [8] vs. the more complex and time consuming, but less interference prone LC-MS methods [9–11].

LC-MS methods are considered more accurate but can also be prone to intra- and inter-laboratory variability. This can be a challenge if the analytical method is developed by the laboratory, where calibrators and controls are prepared in-house, or lyophilized commercial calibrators and controls are used, and are not managed properly. This has been surveyed and outlined in more detail by the International Association of Therapeutic Drug Monitoring and Clinical Toxicology Immunosuppressive Drug Scientific

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Committee [5, 6]. The demand for highly qualified staff is another limitation of LC-MS/MS in a 24/7 clinical analysis environment [7] because of the need for staff trained in complex method development, technical troubleshooting, pharmacology and complex instrument management.

As has been shown before [12], some standardization of the LC-MS results can be obtained by using commercial chromatography immunosuppressant kits including calibrators and controls, and using the same sample preparation protocol on the same LC-MS instrument type. Automated processing of whole blood samples by robotic platforms optimizes workflow further [13], leading to a reduction in manual workload and less risk of errors. An online transfer of the results from the LC-MS instrument to the laboratory information system is an additional measure to reduce errors [14]. However, it is rarely implemented with LC-MS, as manual data control and occasional re-integration of chromatograms not only hinders automatic result acceptance and transfer, but also limits the ability to harmonize result generation.

Recently results from the first fully automated LC-MS/MS method using the Cascadion SM Clinical Analyzer (Thermo Fisher Scientific OY, Vantaa, FI) were published [15]. Using this instrument CsA, TAC, SIR and EVE were quantified in random access mode using primary blood collection tubes without any need for pre-treatment. The whole blood assay does not require any manual intervention and the instrument can be coupled bi-directionally to the laboratory information system (LIS).

To validate the degree of harmonization using this fully automated immunosuppressive drug assay system, three centers across Europe participated in a trial looking at performance differences and analytical bias between different laboratories using this instrument. To distinguish the analytical differences from potential bias introduced pre-analytically (e.g. by aliquoting, transport or sample container differences) each of the three sites collected a sample set including the four immunosuppressants CsA, TAC, SIR and EVE and shared these with the other sites.

Materials and methods

Blood sample collection and study design

Blood samples were collected in ethylenediaminetetraacetic acid tubes from Becton Dickinson (Franklin Lakes, USA) and Sarstedt (Nümbrecht, Germany). Leftover patient samples were used in this trial, for the analysis of CsA, TAC, SIR and EVE. Included in the study were 1097 samples (CsA #411, TAC #433, SIR #114 and EVE #139). These were sent between the Laboratory Medicine of the University Hospital Basel (Switzerland) (USB), the Hospital La Pitié Salpêtrière Paris, (France) (PSL) and Birmingham Heartlands Hospital (United Kingdom) (BHH). The samples were measured immediately with the respective local routine method/instruments directly from the primary sample tube, and were then aliquoted into three polypropylene screw-cap tubes with a volume of at least 0.7 mL each. The aliquots were then frozen immediately at −80 °C. The selection of samples at each center aimed to cover the entire analytical measurement range independent of the transplanted organ.

Unified barcodes were generated to de-identify the aliquot samples but still allow later barcode reading by the fully automated systems at each site, making sample mismatches virtually impossible and ensuring compliance with data protection standards. The sample sets were shipped as single sets on dry ice to the other sites and stored at −80 °C until analysis.

Ethical approval

In all three hospitals, data and samples were all obtained as standard medical care. According to the British, French and Swiss regulation, biobanking and secondary use for scientific purpose of data and human clinical samples are possible as long as the corresponding patients are informed and have not given any objection. According to the British, French and Swiss regulation, no Institutional Review Board approval was required.

Cascadion immunosuppressant assay

The trial was started before completion of the immunosuppressant (ISD) assay CE-IVD registration, but production lots of ISD specific reagents were used at all sites. The sites started with the same lot of calibrators and controls. All consumables and solvents had CE marking as required for *in vitro* diagnostic (IVD) devices.

The reported ranges of results were between the lowest and highest calibrator concentrations (TAC, EVE, SIR approximately 1–30 µg/L; CsA: 10–800 µg/L). The target concentrations of the three quality control levels varied between the different lots but were approximately 2 µg/L, 12 µg/L, and 25 µg/L for TAC, EVE, SIR, and approximately 15 µg/L, 350 µg/L, and 625 µg/L for CsA, respectively.

Precision/accuracy assessment

To demonstrate data comparability across the three sites, all of them determined the imprecision and accuracy of their instrument using the three internal quality control levels provided by the manufacturer. The calibrators and controls were products from Thermo Fisher Scientific Oy (Vantaa, FI). According to the instructions for use and information from the company, they are independently prepared using human source material. The calibrators and controls were referenced to volumetrically prepared reference standards, which in turn were traceable to four recognized LC-MS/MS laboratories. According to the consensus guidelines [5] the acceptance criteria were ≤10% for imprecision, ≤10% for bias and ≤20% for total error, respectively.

Comparison fresh vs. frozen samples

In order to study the influence of frozen samples on the extraction and measurement procedure of the assay, 103 TAC containing patient samples with concentrations between 2 and 24 µg/L were analyzed fresh at one site and thereafter three aliquots of the leftover material prepared. After storage at −80 °C for a minimum of 2 weeks, the aliquots were measured at all three sites. TAC was chosen, because it is the most frequently tested at all sites and is known to be sensitive to degradation at room temperature [16].

Comparison of primary tube vs. secondary cup

The influence of the on-board mixing algorithms for whole blood in primary tubes vs. secondary cups, potentially confounding the analysis, was determined by comparing the results from samples directly measured from primary tubes and additionally from special sample cups at the same site. The sample cups can be used on the instrument for low-volume samples. This functionality, which draws an aliquot of whole blood sample from the 0.5 mL cups, was enabled on the analyzers used during the ring trial ahead of commercial availability.

Comparison of QC data of the three sites during the trial

To assess the degree of potential differences in the performance of the three Cascadion systems under harmonized conditions, the analysis of three concentration levels of QC material was compared and summarized in Box and Whisker plots for calculated bias. QCs were run at the beginning of each day and during the day at intervals according to the requirements from the local regulations. QC materials from two different lots for each control level were used at the Basel, Birmingham and Paris laboratories for 61 days, 49 days and 76 days, respectively.

Method comparisons for the ISD assay

Each instrument contains two chromatography channels, each with separate injection ports, solvent pump and a quick connect cartridge (QCC). Each QCC has a turbo flow column, for sample clean-up, and an analytical column. Despite this integrated multiplexing for increased throughput, none of the analyses distinguished between these channels or was aimed at an even distribution of replicates. QCs did run on both channels while individual samples were injected randomly to one of them. Any potential channel variance is therefore contributing to the total variance between sites.

Statistics

Deming linear regression fit and Bland–Altman analysis plots were created using the Analyse-It package in Microsoft Excel. Additionally, Pearson's correlation coefficient (r) was calculated.

Box and Whisker plots were created using the *Matplotlib* library in Python. They display a representation of the distribution of the determined bias to the respective target or mean concentration. The boxes represent the interquartile distance, and the distance between the first and third quartiles of the distribution. The line inside the box represents the median, and the triangles represent the mean. The dashed line in the box marks the 0% bias and the dashed lines outside the −20% and +20% ranges. The distance from the edge of the box to the whiskers is no greater than 1.5 times the interquartile distance and any circles beyond the whiskers represent the individual results of the distribution. The whisker mark was placed at the data point closest to that limit. No outliers were removed from the datasets.

Results

Repeatability and accuracy

The results of the imprecision and accuracy measurements for the QC materials analyzed at the three sites met the criteria defined above and showed a CV ≤8.2%, a total error ≤19.0%, and a bias <6.7% for the three instruments for all four analytes, respectively. The results were comparable and demonstrated a high harmonization of the immunosuppressants assay and the analysis system (data not shown).

Comparison fresh vs. frozen samples

The comparison of the analysis of TAC in fresh and frozen samples showed a correlation coefficient (r) of 0.992 and a standard deviation of 5.5% (Figure 1). Slope was 0.983 (95% CI: 0.947–1.01) and intercept was 0.126 (95% CI: −0.092 to 0.326). The Bland–Altman plot showed an average bias of only 0.36% (95% CI: −0.70% to 1.42%), with limits of agreement ranging from −10.4 to 11.1%. This demonstrated that freezing and thawing samples for TAC did not have any significant influence on the determined concentration. This is important for comparability of sample analysis either if stored in one place or after shipping to an alternative location using the same type of analyzer. Results from analysis of frozen aliquots were used for the site-to-site comparison.

Comparison of primary tube vs. secondary cup

This comparison, analyzing blood from the low volume sample cup and from the primary sample tube, from patients undergoing TAC treatment (n=59), showed a correlation coefficient (r) of 0.989 and a relative standard deviation of 5.1% (Figure 2). Slope and intercept were 1.030 and −0.1298 with CIs of 0.994, 1.085 and −0.434, 0.08 respectively. The Bland–Altman plot showed an average bias of 1.14% (95% CI: −0.16%, 2.44%), with limits of agreement ranging from −8.9 to 11.1%.

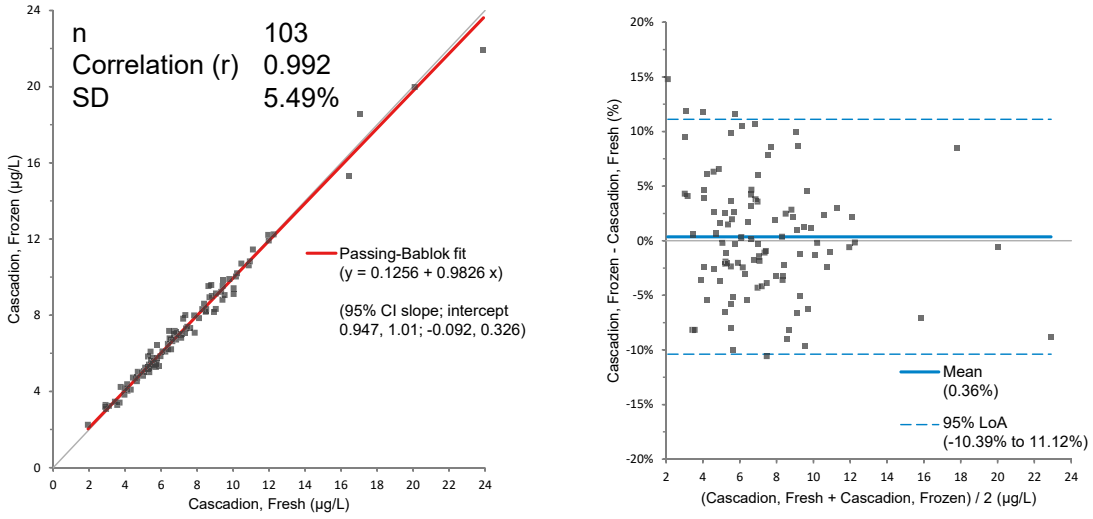

Figure 1: Comparability of fresh vs. frozen samples.
Analysis of tacrolimus samples at one site (USB) shows the degree of comparability of the results after freezing and thawing.

Comparison of QC analysis data from the three sites

The Box and Whisker plots for the three concentration levels of the QC material are shown in Figure 3. The largest negative bias to the known target concentration was observed for EVE Ctrl 1 and SIR Ctrl 3 at Basel, with –3.5% and –3.7%, respectively. The other two Ctrl levels also showed a slightly negative bias for both analytes. The highest positive bias was for EVE Ctrl 3 and TAC Ctrl 3 at Birmingham, at 2.4 and 1.9%, respectively. The number of data points varied between the laboratories because of differences in the local regulatory demands for control frequency.

Analysis of patient samples

Correlations between the analyses of the same patient samples at different sites were determined by comparing the data for the four analytes from each lab with each other lab for all distributed samples. The individual sets sent by each laboratory were also compared separately (Table 1).

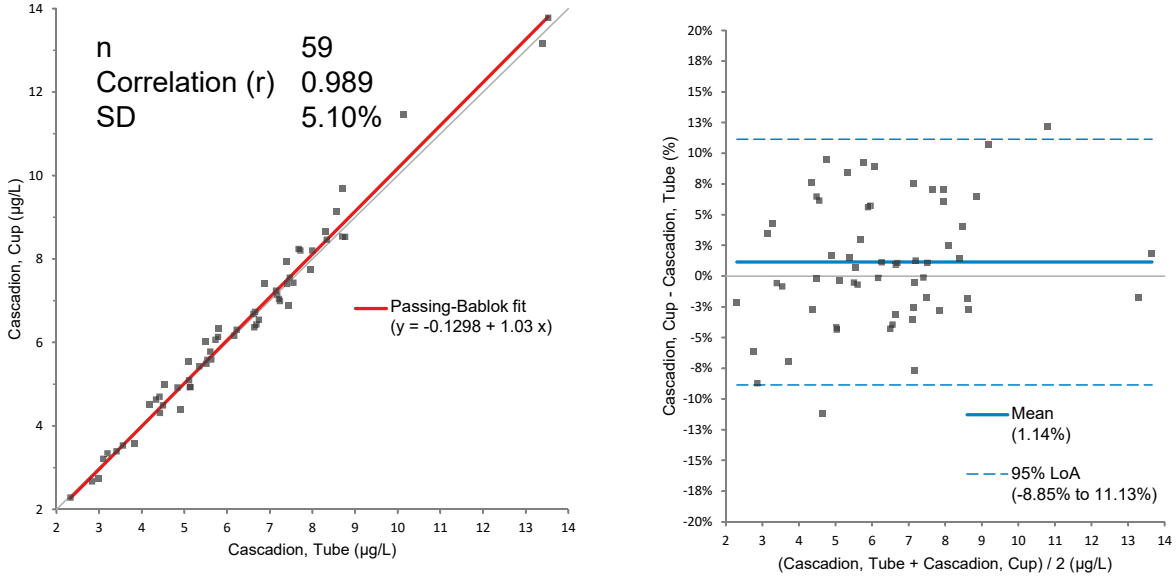

Figure 2: Comparability of primary tube vs. secondary sample cup.
Analysis of tacrolimus samples at one site (USB) shows the degree of comparability of the results from primary tubes as compared to samples aliquoted in secondary sample cups.

The correlation was calculated using the Deming regression analysis. Empty fields indicate that the number of samples collected at the respective sites was too low for statistical analysis. The Pearson's correlation coefficients for all samples with CsA (n=411), EVE (n=139), SIR (n=114), and TAC (n=433) were 0.993, 0.993, 0.993 and 0.990, respectively. For the individual sample sets the lowest correlation was determined for the TAC sample set sent from Basel (n=132) when compared to the Paris and Basel analyses, with an r-value of 0.978. Comparing the analysis of this TAC set, for Basel vs. Birmingham and Birmingham vs. Paris the r-values were 0.993 and 0.986, respectively, indicating a slightly larger deviation at the Paris site.

To further evaluate the degree of deviations between sites, the differences for the individually determined concentrations were calculated as %bias to the mean value of all measurements of the respective patient sample aliquots and summarized in the Box and Whisker plots (Figure 4). The largest deviations were found for SIR with a negative bias of –4.5% at Basel and a positive bias of 2.9% in Paris. CsA measurements had a positive bias of 2.9% at Basel, a negative bias of 1.9% at Birmingham and the smallest interquartile range for the spread of results. The TAC results were closest to the median with a bias ranging from –1.7 to 1.8%. Except for a few outliers, the deviation of results from three different sites was within the ±10% range for all four immunosuppressants and similar to the comparison of fresh vs. frozen samples on an individual instrument.

Discussion

LC-MS/MS based methods for the analysis of immunosuppressive drugs have been established in clinical laboratories for more than 20 years. However, despite mass spectrometry becoming more prevalent in the clinical diagnostics lab and considered to be superior to immunoassays (IA) in terms of specificity and accuracy, IA-based analyzers and assay systems are still used by more than

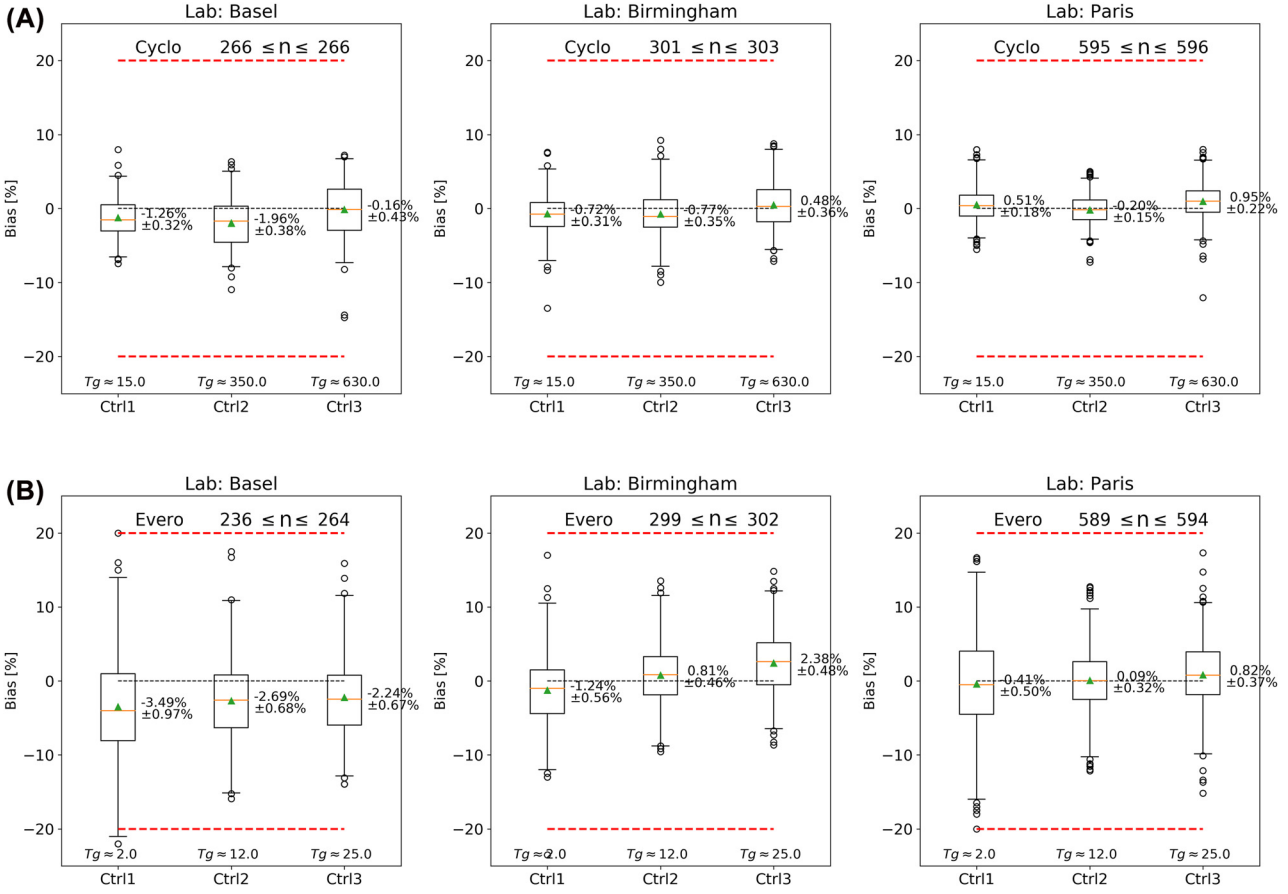

Figure 3: Comparison of QC analysis data at the three sites. Box and Whisker plots for the three concentration levels of the QC material. Bias was calculated based on the assigned values for the respective QCs, which enabled to include the two different lots used.
(A) Cyclo: cyclosporine A, (B) Evero: everolimus, (C) Siro: sirolimus, (D) Tacro: tacrolimus. Tg, target concentration; n, number of replicates.

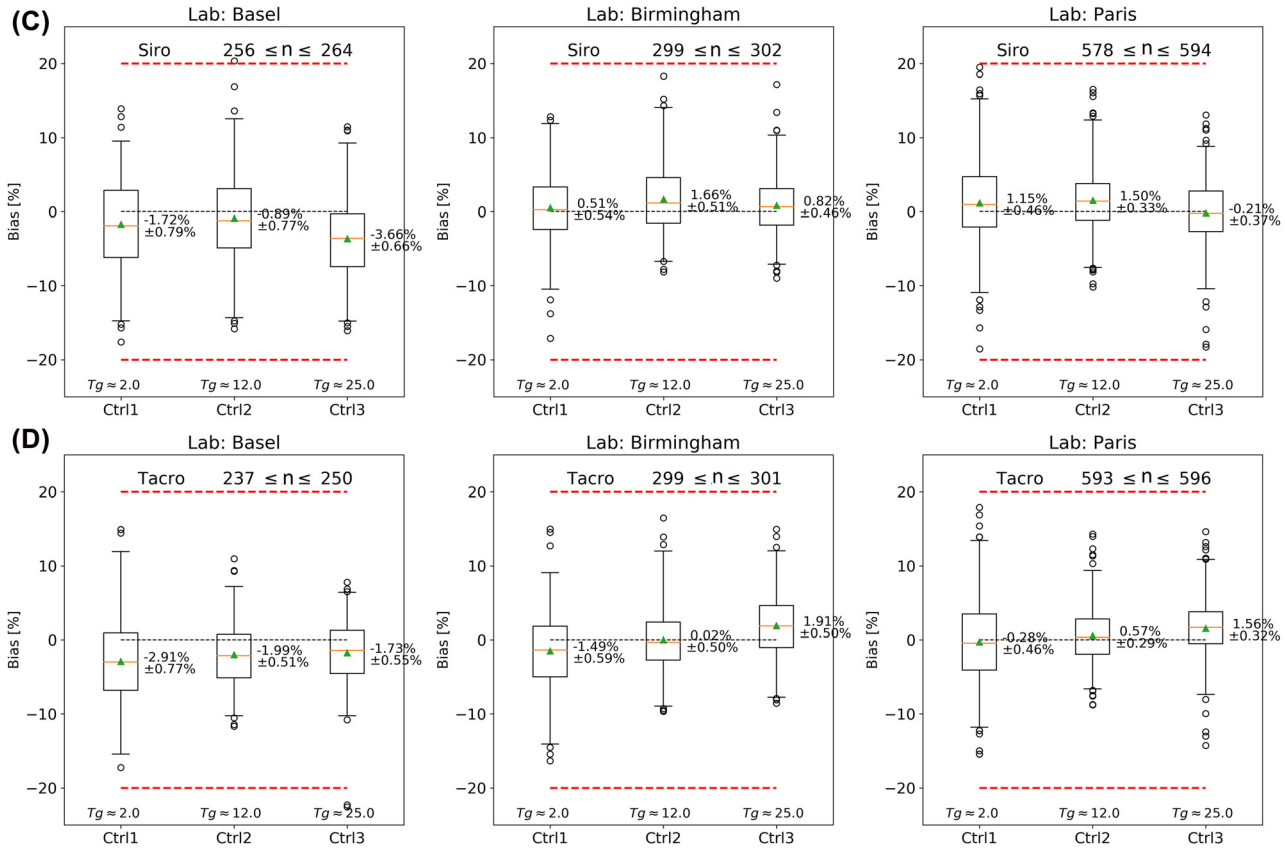

Figure 3: Continued.

half of the laboratories participating in external quality assessment (EQA) schemes in Europe. The Immunosuppressant proficiency testing (IPT) scheme round, March 2021, distributed by LGC showed data from 129 labs using IA and 124 labs using LC-MS/MS, respectively. The benefits to the labs and patient care from timely, 24/7 available, easy-to-use diagnostics and bi-directional LIS integration of IA analyzer partly outweigh the quality improvements achievable by open LC-MS platforms.

The lack of harmonization of LC-MS based instrumentation and methods, random human errors or variability in the sample preparation and data processing, has set certain limits on the uniformity and inter-laboratory comparability of methods and results.

The increased availability of reference materials and well-managed EQA programs have led to general improvements in data quality, method comparability, and patient care in recent years. This enhanced the ability of accurate TDM to reduce or avoid the adverse and toxic effects of inadequate drug dosing.

The availability of IVD-CE-certified LC–MS/MS assays and the current IVD-CE-certified fully automated closed instrument that integrates sample preparation, LC-MS/MS, seamless data processing and bi-directional LIS integration will further improve the adoption and quality of TDM in clinical practice.

Therefore, we assessed the capabilities of the recently released Cascadion SM Clinical Analyzer instrument to reproducibly deliver accurate and comparable results for the analysis of four immunosuppressive drugs in whole blood across country borders and laboratory settings.

The intra-laboratory imprecision (CV) and accuracy (bias) of the analysis were equal or below 8.2 and 6.7%, respectively, for all concentration levels of QC material. This includes variability from both channels of the integrated duplex LC system, and appears entirely satisfactory for the whole blood matrix, representing a benchmark for further comparisons.

For the analysis of ISDs, the storage and transport of frozen samples is not only a matter of analyte stability, but also has a direct impact on the assay. The majority of the-administered drugs such as TAC or SIR are sequestered in erythrocytes [17, 18] and complete hemolysis is required for quantitative analysis. The integrated sample preparation process of the Cascadion ISD assay fulfills this requirement. The very low bias of 0.36% and high

Table 1: Comparison of the analysis of cyclosporine A, tacrolimus, everolimus and sirolimus in patient samples measured at all three sites.

Cyclosporine A						
	USB-BHH		**BHH-PSL**		**USB-PSL**	
	Slope (CI)	**Intercept (CI)**	**Slope (CI)**	**Intercept (CI)**	**Slope (CI)**	**Intercept (CI)**
All (n=411)	0.947 (±0.011)	0.650 (±0.056)	1.014 (±0.014)	−0.084 (±0.066)	0.954 (±0.015)	0.801 (±0.073)
USB (n=93)	0.959 (±0.022)	0.737 (±0.123)	1.011 (±0.033)	0.378 (±0.169)	0.970 (±0.034)	1.092 (±0.179)
BHH (n=150)	0.921 (±0.033)	0.779 (±0.203)	1.046 (±0.035)	−0.513 (±0.212)	0.944 (±0.037)	0.903 (±0.197)
PSL (n=168)	0.961 (±0.013)	0.719 (±0.058)	0.992 (±0.015)	−0.151 (±0.061)	0.953 (±0.017)	0.563 (±0.077)
Everolimus						
All (n=139)	1.025 (±0.029)	0.039 (±0.063)	1.014 (±0.023)	−0.116 (±0.078)	1.051 (±0.023)	−0.091 (±0.076)
USB	[a]	[a]	[a]	[a]	[a]	[a]
BHH	[a]	[a]	[a]	[a]	[a]	[a]
PSL (n=139)	1.024 (±0.029)	0.043 (±0.065)	1.017 (±0.022)	−0.129 (±0.077)	1.053 (±0.023)	−0.098 (±0.078)
Sirolimus						
All (n=114)	1.070 (±0.019)	−0.026 (±0.072)	1.013 (±0.018)	−0.031 (±0.076)	1.092 (±0.024)	−0.058 (±0.087)
USB (n=32)	1.053 (±0.033)	−0.067 (±0.149)	1.031 (±0.031)	0.112 (±0.103)	1.085 (±0.033)	0.044 (±0.136)
BHH	[a]	[a]	[a]	[a]	[a]	[a]
PSL (n=82)	1.078 (±0.022)	−0.017 (±0.074)	1.005 (±0.018)	−0.059 (±0.069)	1.092 (±0.032)	−0.086 (±0.112)
Tacrolimus						
All (n=433)	0.954 (±0.011)	0.058 (±0.056)	1.043 (±0.014)	−0.097 (±0.066)	0.997 (±0.015)	−0.021 (±0.073)
USB (n=134)	0.951 (±0.022)	0.063 (±0.123)	1.053 (±0.033)	−0.146 (±0.169)	0.999 (±0.034)	−0.037 (±0.179)
BHH (n=115)	0.931 (±0.033)	0.019 (±0.203)	1.055 (±0.035)	0.088 (±0.212)	0.992 (±0.037)	0.098 (±0.197)
PSL (n=184)	0.982 (±0.013)	0.028 (±0.058)	1.008 (±0.015)	−0.070 (±0.061)	0.989 (±0.017)	−0.037 (±0.077)

USB-BHH, comparison of the results between the Laboratory Medicine of the University Hospital Basel (Switzerland) and Birmingham Heartlands Hospital (United Kingdom); BHH-PSL, comparison of the results between Birmingham Heartlands Hospital (United Kingdom) and Hospital La Pitié Salpêtrière Paris (France); USB-PSL, comparison of the results between the Laboratory Medicine of the University Hospital Basel (Switzerland) and Hospital La Pitié Salpêtrière Paris (France); CI, confidence interval. [a]Number too small for statistical analysis.

correlation coefficient for TAC concentrations verified that there was no difference in the analysis of fresh and thawed samples. This is beneficial for turnaround times in routine analysis as blood samples can be processed sequentially as they arrive. There is no delay related to pre-treatment in batch mode, such as fast freezing applied with some LC-MS methods or manual addition of lysis reagents as with most IA.

Blood samples of more than 1 mL can be measured directly from the barcoded primary tubes with the on-board mixing tips and a mixing algorithm ensuring homogenization before drawing the aliquot. Low volume samples of at least 300 µL were measured from a sample cup directly after insertion. The comparison of results (tube vs. cup) showed insignificant bias (1.14%) and a high reproducibility between the two routes of introducing samples into the system. Laboratory personnel can use either way as needed and the system software automatically traces and documents the mode of sample introduction. Additionally, the system manufacturer introduced a mixing process for whole blood in low-volume sample cups, with a software update released after the trial. This was not tested, but the respective instructions for use state that up to 20 samples in cups can now be fed into the system at once. This function is important, especially for lower volumes of pediatric samples, but also for the use of primary tube types that have not (yet) been validated with the sample-mixing algorithm.

LC-MS methods with manual sample preparation may have the advantage of being suitable for even lower sample amounts but are prone to variability in pipetting, especially over time [19]. The fully automated system with integrated sample preparation maintains a low variation over weeks and even months. The QC analysis data of the three sites, spanning up to 76 days and including two lots of QCs, verified this by the low relative bias to assigned target concentrations independent of the concentration level or the analyte. As expected CsA exhibited the narrowest range

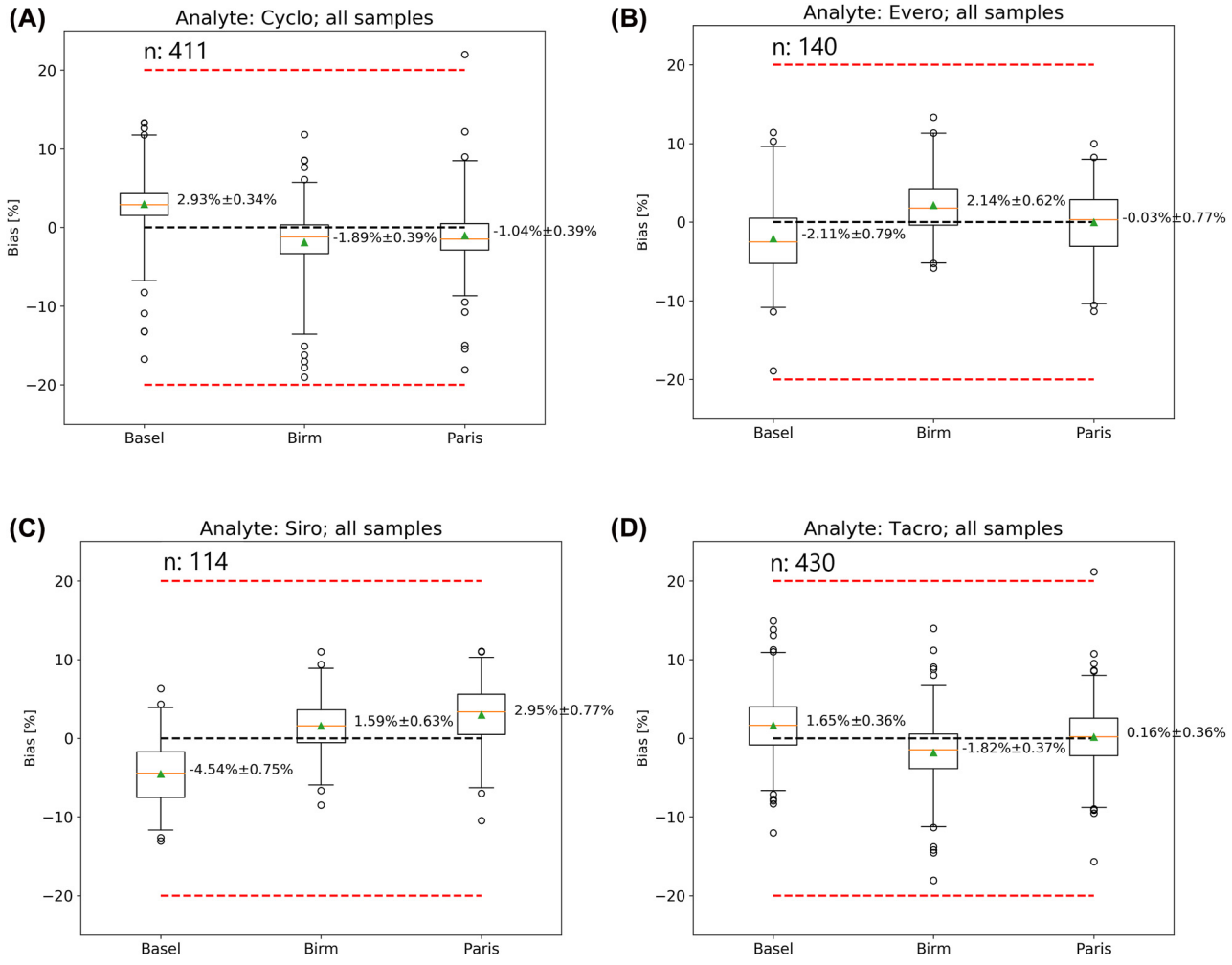

Figure 4: Comparison of sample analysis data of the three sites Box and Whisker plots of all measurements of the respective patient sample aliquots as %bias to the mean value at the three sites. Data include the three sample sets exchanged between the sites.
(A) Cyclo: cyclosporine A, (B) Evero: everolimus, (C) Siro: sirolimus, (D) Tacro: tacrolimus.

of bias. The higher dose concentration (approximately 10 times) and higher signal intensity in the mass spectrometric detection enables reproducible data processing. In addition, the software algorithm for the fully automated integration of LC-MS peak areas (for both, internal standards and analytes) for determination of the analyte concentration based on isotope-dilution mass spectrometry appeared very robust and reproducible at low concentrations. The distribution of individual bias was symmetrical and within the assay measurement uncertainty for the three concentration levels of all analytes at the three sites. No systematic analytical deviations or instrument-specific variability were observed.

While typical QC materials are pre-processed and well homogenized during bulk manufacturing, the pipetting, processing, and analysis of individual patient blood samples provide significantly more challenges, e.g. due to undetected clots, insufficient blood cell lysis or mixing and matrix effects. Pre-analytical steps (sample collection, aliquoting, and transport), sample containers (materials) from different manufacturers or thawing regimes at each lab may introduce systematic or random variability. Despite all of these variables, comparing correlations of the measurements of the individual sample sets at the three sites showed only marginal differences (Table 1). This indicated a careful and thorough preanalytical sample handling in the laboratory at all sites and showed the high precision of the three instruments for each individual sample analysis. Thus, even slight differences between the sample sets becomes detectable. For instance, the TAC sample set sent by Basel, showed a small measurement deviation relative to the Paris site (r=0.978). While Birmingham and Basel correlated very well (r=0.993) the

Birmingham and Paris correlations were in between (r=0.989). Other analytes in this sample set did not show such a deviation, and an effect from transport, storage, or thawing was assumed. There would not have been any clinical relevance of these subtle differences, but it indicated the high interlaboratory analytical comparability achieved with the fully automated LC-MS-based systems.

The site specific mean analytical bias, assessed by comparing the individual patient sample values to the mean of all sites (Figure 4), was below 5% for all sites and analytes. There was no pattern whereby sites measured constantly higher or lower than other sites, and the remaining bias may be mainly attributed to the measurement uncertainty in the generation of the calibration curves of the analytes. In our study, the immunosuppressant assay calibration was required weekly by the instrument. Later, the calibration curve stability was extended to 30 days by the manufacturer, which will probably further increase the reproducibility of the assay. Although the whole blood matrix processed was fully automated, the spread of the individual values for the patient samples was in the same range as that observed with the homogeneous control materials.

Seger et al. indicated in 2016 [5] that the interlaboratory imprecision is significantly higher than the intra-laboratory imprecision for a specific method and estimated as an example of 20% vs. 5%. With the fully automated LC-MS/MS based Cascadion system inter- and intra-laboratory variability were almost equally low for the ISD analysis of whole blood.

One limitation of this study is the missing dilution experiment of samples having a concentration above the upper limit of quantification. During the study period, only very few samples (n=15) had concentrations above the calibration range requiring dilution, making it impossible to generate statistically valid data. Another limitation is the missing study on trueness of the results of the Cascadion. All sites have successfully participated at external quality control schemes for their routine method/instruments during the study. In parallel, the external quality control samples have additionally been measured with the Cascadion instruments. According to the data provided by the EQA provider, all results obtained were well comparable with other LC-MS/MS methods.

Overall, the outcome of the study demonstrates a new level of result harmonization for LC-MS/MS based immunosuppressant analysis with a commercially available fully automated platform for routine clinical application. As ISDs have defined molecular weights and known structures, standardized analysis should be an overall aim in the near future.

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Competing interests: Noël Zahr and Katharina M. Rentsch have received speaker's honoraria from Thermo Fisher.

Informed consent: Not applicable.

Ethical approval: Not applicable.

Data availability: The datasets generated during the current study are available from the corresponding author on reasonable request.

References

1. Brunet M, van Gelder T, Asberg A, Haufroid V, Hesselink DA, Langman L, et al. Therapeutic drug monitoring of tacrolimus-personalized therapy: second consensus report. Ther Drug Monit 2019;41:261–307.
2. Shipkova M, Hesselink DA, Holt DW, Billaud EM, van Gelder T, Kunicki PK, et al. Therapeutic drug monitoring of everolimus: a consensus report. Ther Drug Monit 2016;38:143–69.
3. Yatscoff RW, Boeckx R, Holt DW, Kahan BD, LeGatt DF, Sehgal S, et al. Consensus guidelines for therapeutic drug monitoring of rapamycin: report of the consensus panel. Ther Drug Monit 1995; 17:676–80.
4. Oellerich M, Armstrong VW, Kahan B, Shaw L, Holt DW, Yatscoff R, et al. Lake Louise Consensus Conference on cyclosporin monitoring in organ transplantation: report of the consensus panel. Ther Drug Monit 1995;17:642–54.
5. Seger C, Shipkova M, Christians U, Billaud EM, Wang P, Holt DW, et al. Assuring the proper analytical performance of measurement procedures for immunosuppressive drug concentrations in clinical practice: recommendations of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology Immunosuppressive Drug Scientific Committee. Ther Drug Monit 2016;38:170–89.
6. Christians U, Vinks AA, Langman LJ, Clarke W, Wallemacq P, van Gelder T, et al. Impact of laboratory practices on interlaboratory variability in therapeutic drug monitoring of immunosuppressive drugs. Ther Drug Monit 2015;37:718–24.
7. Vogeser M, Zhang YV. Understanding the strategic landscape surrounding the implementation of mass spectrometry in the clinical laboratory: a SWOT analysis. Clin Mass Spectrom 2018;9: 1–6.

8. Dasgupta A. Impact of interferences including metabolite cross reactivity on therapeutic drug monitoring results. Ther Drug Monit 2012;34:496–506.
9. Ward G, Simpson A, Boscato L, Hickman PE. The investigation of interferences in immunoassay. Clin Biochem 2017;50:1306–11.
10. McShane AJ, Bunch DR, Wang S. Therapeutic drug monitoring of immunosuppressants by liquid chromatography-mass spectrometry. Clin Chim Acta 2016;454:1–5.
11. Taibon J, van Rooij M, Schmid R, Singh N, Albrecht E, Anne Wright J, et al. An isotope dilution LC-MS/MS based candidate reference method for the quantification of cyclosporine A, tacrolimus, sirolimus and everolimus in human whole blood. Clin Biochem 2020;82:73–84.
12. Annesley TM, McKeown DA, Holt DW, Mussell C, Champarnaud E, Harter L, et al. Standardization of LC-MS for therapeutic drug monitoring of tacrolimus. Clin Chem 2013;59:1630–7.
13. Marinova M, Artusi C, Brugnolo L, Antonelli G, Zaninotto M, Plebani M. Immunosuppressant therapeutic drug monitoring by LC-MS/MS: workflow optimization through automated processing of whole blood samples. Clin Biochem 2013;46: 1723–7.
14. Zhang YV, Rockwood A. Impact of automation on mass spectrometry. Clin Chim Acta 2015;450:298–303.
15. Horber S, Peter A, Lehmann R, Hoene M. Evaluation of the first immunosuppressive drug assay available on a fully automated LC-MS/MS-based clinical analyzer suggests a new era in laboratory medicine. Clin Chem Lab Med 2021;59:913–20.
16. Kaplan I, Yuksel H, Evliyaoglu O, Basarali MK, Toprak G, Colpan L, et al. Effects of storage temperature and time on stability of serum tacrolimus and cyclosporine A levels in whole blood by LC-MS/MS. Int J Anal Chem 2015;2015:956389.
17. Peters DH, Fitton A, Plosker GL, Faulds D. Tacrolimus. A review of its pharmacology, and therapeutic potential in hepatic and renal transplantation. Drugs 1993;46:746–94.
18. Yatscoff RW. Pharmacokinetics of rapamycin. Transplant Proc 1996;28:970–3.
19. Vogeser M, Kirchhoff F. Progress in automation of LC-MS in laboratory medicine. Clin Biochem 2011;44:4–13.