

The impact of laboratory practices on inter-laboratory variability in therapeutic drug monitoring of immunosuppressive drugs. Christians U¹, Vinks S², Langman L³, Clarke W⁴, Marquet P⁵, Wallemacq P⁶, Van Gelder T⁷, Renjen V⁸, Meyer EJ⁹. ¹University of Colorado, Aurora, CO, USA; ²Cincinnati Children's Hospital and Medical Center, Cincinnati, OH, USA; ³Mayo Clinic, Rochester MN, USA; ⁴John Hopkins School of Medicine, Baltimore, MD, USA; ⁵University Hospital of Limoges, Limoges, France; ⁶Cliniques Universitaires St Luc, UCL, Brussels, Belgium; ⁷Erasmus Medical Center, Rotterdam, The Netherlands; ⁸Navigant Consulting Inc., New York, NY, USA; ⁹Novartis Pharmaceutical Corp., East Hannover, NJ, USA

Background

The immunosuppressant drugs utilized for the prophylaxis of organ rejection in solid organ transplantation are in many cases narrow therapeutic index drugs, and as such require therapeutic drug monitoring (TDM). Administration of these agents, within the suggested therapeutic range, is critical to patient care [1-4]. Long term graft survival, especially in renal transplant patients, due to the inherent nephrotoxicity of immunosuppressant drugs such as cyclosporine and tacrolimus, is dependent on the proper administration of these immunosuppressive agents. Over and under-immunosuppression can negatively impact the safety and efficacy of post-transplant treatment regimens, respectively. Immunosuppressant drug exposure well above the recommended therapeutic ranges can lead to a multitude of adverse events such as infection, malignancy, cardiovascular disease, diabetes, proteinuria, hyperlipidemia, peripheral edema; while sub-therapeutic concentrations can result in graft loss due to acute and/or chronic rejection. Even with immunosuppressant drug concentrations within the recommended target range, transplant physicians will often make adjustments to achieve a tradeoff between rejection and infection, or other side effects. This is especially important when multiple immunosuppressant drugs are used concomitantly.[5] Reliable, accurate and precise test methods are therefore essential in order to effectively monitor levels and to make proper dose adjustments when, and if, necessary. Not only is it important to have consistency within the patient's transplant center's laboratory, but controlling between laboratory variability is particularly important since patients may utilize multiple testing locations, and different assays, throughout the lifecycle of their transplant [6-8].

Multiple assays, with differing formats and procedures, are currently used to assess the blood levels of immunosuppressant drugs. Large variability has been reported in the accuracy, precision, specificity, sensitivity, and reporting ranges of these assays. This variability is seen with both of the main assay technologies utilized in immunosuppressant drug TDM, liquid chromatography tandem mass spectroscopy (LC-MS/MS) (primarily laboratory developed tests) and immunoassays (IA) (primarily commercial assays). In fact, in proficiency studies as much as 40-45% variability has been observed in reported results from participating laboratories with tacrolimus [7,8], the most common of the immunosuppressant drugs tested. This same level of between laboratory variability has been observed with everolimus in smaller studies [9]. This relatively high level of between laboratory variability suggests that the integrity and utility of the results are impacted, and that there are likely multiple contributing factors.

Surprisingly, there have only been very few attempts to study the causes underlying between laboratory variability. The aim of this study was to systematically document current practices utilized for immunosuppressant drug TDM in clinical laboratories and identify methodological and practical differences which may be the basis for the variability observed between laboratories. Multiple aspects of the design, development, validation, implementation, and quality control of immunosuppressant drug TDM methods were assessed.

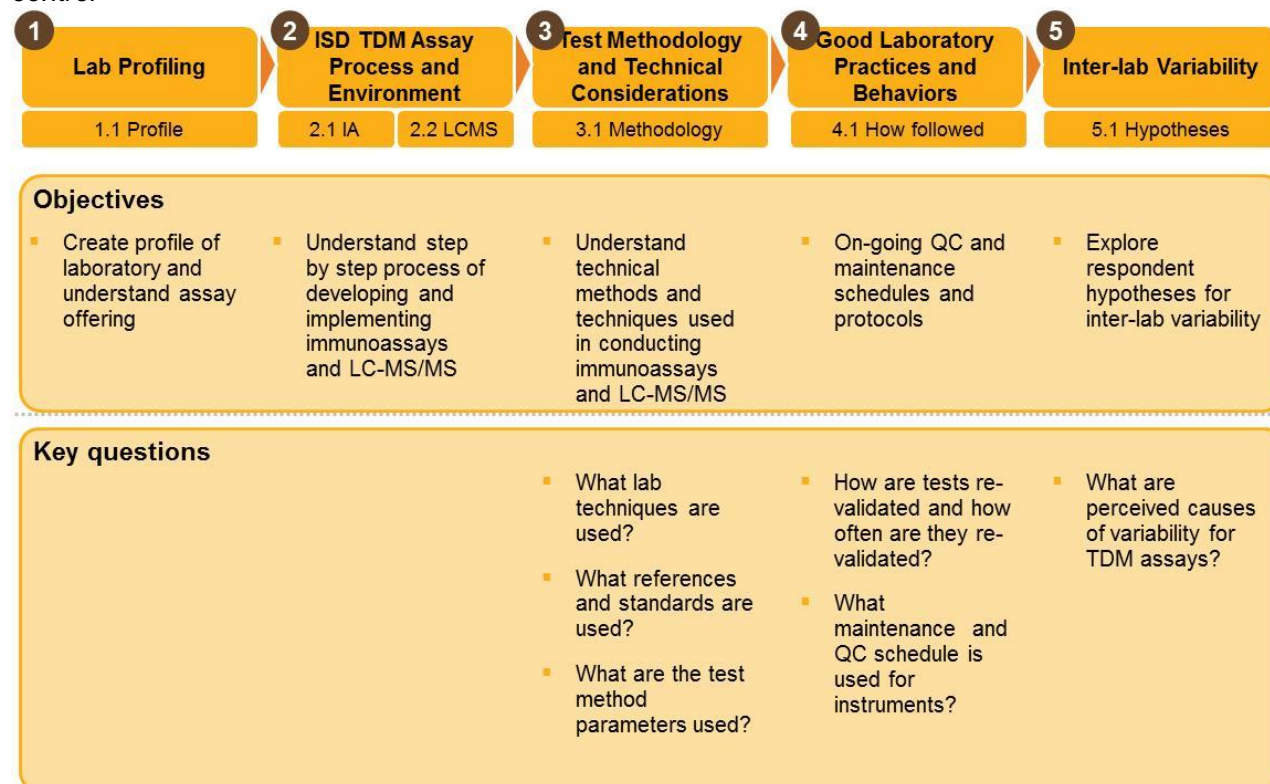
Methods and Survey Tool

The data collection was primarily conducted *via* a web-based survey under the Codes of Conduct for Market Research. To ensure the confidentiality of all participants, Navigant Consulting managed all aspects of the study including survey execution and compilation of results. The survey was sponsored by Novartis and participation was fully endorsed and supported by the International Association of Therapeutic Drug Monitoring and Clinical Toxicology. Invitations to participate in the survey were distributed to clinical laboratories providing immunosuppressant drug TDM for transplant centers in 17 countries. Eligible respondents had to confirm their technical knowledge and experience in developing and performing immunosuppressant drug TDM assays, or in supervising the same.

The survey was comprehensive, and covered assay development as well as the entire assay testing process, from sample acquisition to results reporting. The instrument consisted of 128 questions, organized into 8 sections, and took on average 90 minutes to complete. General, as well as specific, questions related to immunoassays, LC-MS/MS, and high-performance liquid chromatography with ultra-violet detection (HPLC-UV) were included. The survey structure is depicted in Figure 1.

Figure 1. Schematic overview of survey structure, flow and topics.

Abbreviations: IA – immunoassay, ISD – immunosuppressant drug, TDM – therapeutic drug monitoring, LC-MS or LC-MS/MS – liquid chromatography tandem mass spectroscopy, QC – quality control



Specifically the following areas were assessed:

- Sample collection, shipping, and storage methods and parameters
- Sample extraction methods / parameters
- Assay technology and associated equipment
- Assay methods / steps / parameters
- Analysis methods / parameters

- Results reporting (e.g. reporting ranges vs. validated ranges)
- Assay performance specifications (e.g. accuracy, precision, sensitivity, specificity, linear range, lower limit of detection [LLOD], lower limit of quantitation [LLOQ], upper limit of detection [ULOD], upper limit of quantitation [ULOQ]) and validation practices
- Environmental / quality control procedures
- Choice of controls, calibrators, and reference standards
- Respondent perceptions of sources of variability

Results were compiled and analyzed in aggregate. Country specific sub-analyses were not performed due to the low participation rate outside of the USA and France (Table 1).

Results

The survey was fielded from mid-April through mid-July 2013. Surveys were completed by 76 laboratories in 14 countries.

Table 1. Participant numbers with complete survey entries broken down by country.

Country	Total Completions (n=76)
Australia	5
Brazil	3
France	15
Germany	4
Italy	1
Spain	5
USA	29
Belgium	2
Netherlands	2
Taiwan	0
Chile	4
Venezuela	0
UK	1
South Africa	0
South Korea	1
Thailand	3
Japan	1

There were differences in sample handling and storage. Laboratories received samples from local, regional and national sources and time periods from sample collection to analysis varied from several hours to one week. Many laboratories did not control for temperature during this time period. Fifteen % of the laboratories did not seem to have specific procedures for temperature control of samples during transport and storage at all. A large percentage of laboratories did not reject samples due to lack of perceived issues with sample clotting (29%), incorrect anticoagulant used during sample acquisition (43%), or requirements of minimal sample volumes not met (53%). Sample preparation was mostly manual (72%). The extraction procedures were often poorly controlled and not standardized. Thus, only 25% of the laboratories reported that the temperatures during centrifugation steps were well controlled.

In terms of LC-MS/MS assays, HPLC separation varied greatly and 6% of the laboratories even used flow injections. Although isotope-labeled internal standards are considered state-of-the-art for LC-MS/MS assays, 62% of the laboratories still used ascomycin as internal standard for tacrolimus analysis, and in some cases also for the quantification of structurally less related compounds such as sirolimus (29%) and cyclosporine (6%). There was marked variability in terms of the storage conditions and expiration dates of reference material stock solutions.

Stock solutions for the preparation of calibrators were also used for quality control samples by 34% of the laboratories. In addition, 25% of the laboratories used serial dilution for the preparation of their calibrators. Depending on the immunosuppressant, in 19-28% of the laboratories the calibration curves were based on 3 or less non-blank samples.

Assays based on commercially purchased kits were less likely to be validated by the laboratory than in-house developed assays. While approximately 80% of the laboratories using commercial assays established linearity, intra- and inter-day imprecision and accuracy, only relatively few of such LC-MS/MS laboratories assessed matrix effects (ion suppression, 28%), carry-over (57%) and/or autosampler stability (20%) in their specific laboratory using their specific equipment. In-house developed LC-MS/MS assays also did not seem to always be sufficiently validated. Only 89% of such laboratories reported that they had tested for matrix effects, only 80% had tested for carry-over and only 67% for autosampler/extracted sample stability. System suitability testing was carried out by 73% of the laboratories every six months or less frequently. No system suitability testing was conducted by 9% of the laboratories. Once established, between 35% (LC-MS/MS) and 45% (immunoassay) of the laboratories had not revalidated their assay over the years. Twelve % of the laboratories did not seem to participate in any kind of proficiency testing program and had not cross-validated their results with other laboratories.

Experience level of the analytical personnel running samples, which is critical for LC-MS/MS assays, varied. The minimum experience level of individuals ranged from 0-3 months to over one year. On an average, individuals were only moderately experienced to perform the technique. Only 4% of the laboratories had monthly training sessions and 71% trained personnel only "as necessary" and/or as part of a corrective action. In 4% of the laboratories there was no training policy in place at all. Instead of prospectively maintaining an adequate educational and training level, many laboratories only re-train personnel "after the fact" once problems have occurred.

Discussion

Unfortunately, it was only possible to briefly summarize key results here. In this survey of 76 centers in 14 countries performing TDM of immunosuppressant drugs, multiple potential issues were identified and common themes emerged. For LC-MS/MS and immunoassay laboratories there were common, as well as very different, issues identified, largely due to the fact that LC-MS/MS are pre-

dominantly laboratory-developed tests (79% of all LC-MS/MS assays reported) whereas immunoassay systems are commercially provided.

The most prevalent and likely contributing causes to between laboratory variability appear to be:

- lack of proper technique for sample preparation and handling,
- lack of QC of patient samples upon receipt,
- lack of, or insufficient, validation of assays (more for IA),
- improper preparation and use of calibrators and controls (for LC-MS/MS) (e.g. insufficient number of calibrator levels in a calibration curve, use of same stock solution for both calibrators and quality controls),
- use of improper reference standards (for LC-MS/MS),
- poor compliance with what is generally considered good laboratory practices,
- insufficient personnel training standards.

Thus, the results of our survey suggest that there are three main reasons for between laboratory variability: 1) the lack of standardization of laboratory procedures and workflows starting with sample collection and handling, 2) the lack of use of appropriate reference materials (e.g. isotope-labeled internal standards for LC/MS-MS) and samples (calibrators and quality controls), and 3) poor compliance with internationally accepted good laboratory practice guidelines (e.g. quality control, quality assurance, validation, training of personnel). In this context, it is recognized that this was a global survey and that minimum regulatory requirements are different in different parts of the world. Hence, it will be critical to develop consensus documents describing best practices for the therapeutic drug monitoring of immunosuppressants in detail. In addition, the availability of standardized reference materials and samples, as well as their generally accepted use, will likely result in the improvement of inter-laboratory variability. [7,8] Current consensus documents have focused mostly on clinical aspects of immunosuppressant drug TDM [9-11]; however, our survey suggests that consensus on the technical level will be of great importance to improve quality and between laboratory comparison, as well as portability of results. Based on such technical consensus, appropriate educational and training materials, that can be widely distributed, should be developed.

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