Liquid Chromatography–Tandem Mass Spectrometry or Automated Immunoassays:
What Are the Future Trends in Therapeutic Drug Monitoring?

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Therapeutic drug monitoring (TDM)6 of certain drugs with a narrow therapeutic index significantly improves patient outcome. For example, the quantification of immunosuppressive drugs in samples from patients after organ transplantation is an essential prerequisite for the prevention of both adverse drug reactions and rejection events. The need for accurate, precise, and standardized measurement of drugs presents a major challenge for clinical laboratories and the diagnostics industry. A plethora of different techniques have been developed in the past to meet these requirements. Currently, liquid chromatography–tandem mass spectrometry (LC-MS/MS)-based methods and immunoassays appear to be the most prevalent approaches in clinical laboratories. Since these techniques differ in many aspects, in making the choice the laboratory must consider the technical, clinical, and economic criteria, as well as staff qualifications.

In this Q&A, 4 leading experts in the field of immunosuppressive drug monitoring present their opinions on the advantages and disadvantages of the analytical methods and provide a general guide for the optimization of analytical strategies in the field of TDM.

From your perspective, what are the advantages and disadvantages of mass spectrometry–based assays and immunoassays in the context of TDM?

Gregory Maine: One of the main advantages of mass spectrometry is that users can develop their own assays. Mass spectrometry–based assays can be analytically sensitive, specific, and capable of measuring several compounds in a single run. The disadvantages of this technique include that the instrument is not a “turnkey” analyzer, it requires large capital investment and service costs, it is subject to matrix effects and ion suppression, and the results from different laboratories are not necessarily comparable.

The advantages of drug immunoassays include their easy integration into the core laboratory and the ability to measure them along with other immunoassays, such as those for serologic factors and hormones. In addition, TDM immunoassays from the same manufacturer share the same method, calibrators, and instrumentation, resulting in de facto harmonization between laboratories. The main disadvantage is the fact that these assays can be affected by cross-reactivity with metabolites and other substances.

Paul Taylor: Both technologies have their respective strengths and weaknesses for use in TDM. Tandem mass spectrometry has a distinct advantage over immunoassays in that this technology provides the ability to measure multiple analytes in one run. This is a cost-effective approach to monitoring patients who are receiving multidrug therapy (e.g., antiviral therapy for HIV patients). The exquisite selectivity provided by successive mass filtrations is another advantage of tandem mass spectrometry over immunoassays, as is shown for immunosuppressant drugs. Another distinct advantage of mass spectrometry is the ability to develop methods in a more rapid...
time frame and the associated autonomy of not having to rely on an immunoassay manufacturer to provide a new product. An example would be the change to 2-h postdose monitoring for cyclosporine. Analysts were able to readily adapt their mass spectrometric methods to cater to the monitoring of these potentially higher concentrations, while those analysts using immunoassays relied on dilution protocols until the commercial manufacturers modified their products.

Immunoassays have excellent automation, a major weakness of mass spectrometry. The simplicity of automated immunoassays provides ease of use, while mass spectrometry methods require specialized trained scientific staff. This can become even more important when “out of normal hours” testing of patients is required. Another major advantage of immunoassays is the ability to perform random-access testing. Further, immunoassays are provided as a complete solution for the monitoring of a particular drug, while mass spectrometric methods require the scientist to establish a method that might require the sourcing of certified materials and internal standards.

Gerhard Veen: Evaluation of technologies should be based on medical value and the benefits they offer to patients. In the case of TDM, analytical accuracy and sample turnaround time (TAT) are the highest priorities, due to the importance of maintaining therapeutic values within a narrow, predefined range. Although the TATs are comparable for the 2 techniques, immunoassays offer far greater throughput than LC-MS/MS. The 2 techniques also produce comparably reliable results, although the fact that automated immunoassay systems do not rely on dedicated and specialized operators removes a potential source of human error and site-to-site or period-to-period variability.

Pierre Wallemacq: This is a difficult question because the answer may quickly change over time due to the rapid progress in both techniques. TDM analyses, as with many other analyses, are expected to meet several current requirements: (a) integration in a core laboratory (which entails increased automation), increased consolidation (performing more tests on fewer platforms), and reducing operating costs; (b) traceability for accreditation and method validation; and (c) analytical performance in terms of sensitivity, specificity, imprecision, accuracy, possible analytical interferences, robustness, and consistency.

Generally, mass spectrometric methods are considered reference methods, whereas immunoassays are leading in their capacity to be better integrated into a core laboratory (automation, less troubleshooting) and in providing validated kits and methods [European Conformity (CE) label or US Food and Drug Administration (FDA) clearance]. However, impressive efforts have been put forth by the manufacturers of immunoassays and chromatographic equipment to improve their respective weaknesses. For instance, some LC-MS/MS in vitro diagnostics kits are now commercially available with CE labels and FDA clearance, and some immunoassays display an analytical performance (sensitivity, specificity, accuracy) that is equivalent to, or even better than, LC-MS/MS techniques. Unfortunately, some immunoassays have poor analytical performance, and some poorly validated LC-MS/MS methods display unacceptable performance (e.g., ion suppression effects), with both resulting in inconsistent results with potential clinical impact.

Briefly, the advantages of mass spectrometry include the potential analytical specificity and sensitivity, multiplexing measurements (simultaneous quantification of several drugs), flexibility, relatively high throughput, and potential for important cost savings. The disadvantages include a lack of automation, the need of rigorous validation (avoid ion suppression effects), a certain lack of robustness, a lack of a 7-days-per-week technical support service, and the need for qualified staff.

For immunoassays, the advantages include automation and integration into a core laboratory, better traceability (online to a laboratory information system, bar code readers, and well-validated kits), no need for specialized personnel, robustness, and affordable maintenance contracts that include 7-days-per-week technical support. The disadvantages include a relatively poor analytical performance—at least for some assays—such as calibration bias, analytical sensitivity and specificity with a number of potential interferences (cross-reactivity with other drugs or metabolites, heterophilic antibodies, endogenous compounds), and cost of reagents.

In your opinion, what will be the leading technology in the future?

Gregory Maine: Both methods will continue to be in use and remain competitive due to the different needs and capabilities of individual laboratories (number of samples, qualified staff, budget, laboratory facility,
results-TAT requirements). Assay accuracy and imprecision, comparability of results between laboratories, full automation, and laboratory cost will be key drivers in future technologies.

There will be a desire to standardize, or at least harmonize, testing as the electronic medical record becomes more prevalent and healthcare providers try to monitor patients without taking into account fluctuations in results due to analytical discordance among methods.

**Paul Taylor:** The current leading technology for TDM is immunoassays, and this should continue into the near future. Tandem mass spectrometry has found a niche in areas such as monitoring immunosuppressant and antiretroviral drugs, but overall it is not the dominant technology. Tandem mass spectrometry may also find a role in the evolution of pharmacodynamic monitoring. Unfortunately, there are no published clinical data that clearly show that one technology provides results that lead to better patient outcomes. Such studies would be a major driver in moving toward that “clinically superior” technology in the future.

Immunoassays, with their innate ability to “find a needle in the haystack,” and tandem mass spectrometry, with its selective detection based on the physicochemical properties of the analyte, are complementary methodologies. The combination of these technologies into a hybrid instrument would be a fantastic marriage and may be the future of TDM.

**Gerhard Veen:** Public and private healthcare systems are increasingly seeking to pay for value and outcomes rather than for volume or number of diagnoses, and therefore the technology most likely to be widely adopted will be the one offering the greatest medical value in terms of benefits to patients, healthcare professionals, and payers. Automated immunoassay is currently the leading technology used in laboratories providing TDM and in the near future is therefore likely to be the leading technology in terms of widespread use.

**Pierre Wallemacq:** We observed an impressive increase in LC-MS/MS users in TDM, as displayed by external proficiency-testing schemes. Most likely, this progress will keep going because of increased penetration into the large and very large centers, but I don’t believe LC-MS/MS will replace immunoassays in laboratory medicine. Immunoassays will probably remain the leader, but with a reduced advantage.

### What are the implications of automation and work flow improvement?

**Gregory Maine:** High-throughput LC-MS/MS methods using rapid chromatography are available, but they may not be suitable for routine use and are more susceptible to matrix effects. There is a high-throughput tacrolimus immunoassay with automated on-board pretreatment; however, this assay has been plagued by endogenous antibody interference, rheumatoid factor interference, and high assay imprecision at low concentrations. All strategies for automation, especially for assays requiring whole blood, should include a robust automated extraction step that minimizes matrix effects in LC-MS/MS and heterophile antibody interference in immunoassays.

**Paul Taylor:** As previously mentioned, immunoassays have the ability to provide random-access testing. This is a major advantage in a laboratory providing a variety of drug assays (often required on a fast TAT) for relatively small numbers of patients. Currently, LC-MS/MS lacks this flexibility and is really suited to high-volume drug tests. As an example, in our laboratory we perform over 20,000 tacrolimus tests per year with a dedicated tandem mass spectrometer. This batch-analysis approach provides clinicians with timely results in an efficient and price-competitive manner. To interrupt this high-throughput work flow to perform another type of drug test is currently inefficient, difficult, and problematic.

Excellent automation of immunoassays is available. This, coupled with the flexibility of the instrumentation, makes this technology superior to tandem mass spectrometry in terms of work flow. Currently, online sample preparation (typically column switching) is the basis for automation in LC-MS/MS. Automation of mass spectrometry methods must be greatly improved. The ultimate in automation would be sampling from the primary blood-collection tube, random-access sample preparation (possibly a generic method), and, finally, direct reporting from the mass spectrometer. Until this is available, LC-MS/MS cannot compete with immunoassays on the issue of automation.

**Gerhard Veen:** The most obvious implication and benefit of increasing automation is the reduction in the risk of human error and overall costs. In addition, streamlining work flow also results in immediate medical value by expediting clinical decision-making through reduction in sample TAT. This in turn leads to earlier and more highly informed treatment decisions that help to maximize therapy efficacy and reduce empirical treatment. In the case of TDM, much of the testing will be routine, and therefore the potential to expand the level of automation is likely to be desirable in the majority of settings.

**Pierre Wallemacq:** Automation is mandatory in the current context of budget restriction and staff reduc-
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Pierre Wallemacq: Each laboratory should draw up a business plan integrating a number of items, such as (a) number of analyses per year, (b) differential reimbursement (immunoassay vs LC-MS/MS), (c) costs and depreciation of instruments, (d) reagent costs, (e) maintenance contracts, (f) staff numbers and qualifications, and (g) breakdown and backup. The result of this business plan will obviously vary from one center to another and from one country to another, and should not be generalized.

For large series of analyses, the LC-MS/MS technique most likely has an advantage because of the reagent cost savings. However, the decision to use in vitro diagnostics LC-MS/MS kits, deuterated internal standards, and solid-phase extraction may significantly reduce this advantage, resulting in a very close economic balance and leaving the decision to be based on analytical and medical performance.

What are appropriate strategies to ensure high quality and the necessary standardization of these assays?

Gregory Maine: A reference measurement system for each drug should be developed, and should consist of an internationally recognized reference material and reference method, such as exact matching-isotope dilution mass spectrometry, which employs an isotopically labeled internal standard. Immunoassay quality can be improved by the development of assay protocols and new antibodies for better detection of the parent drug compound and minimization of cross-reactivity with metabolites. LC-MS/MS assay quality can be improved by the development of stable isotopically labeled internal standards for better control of matrix effects and ion suppression. Robust automated sample extraction will improve the quality of both methods.

Paul Taylor: The measurement of internal and external quality controls should be used to confirm the quality of results produced by either technology. Laborato-

Gerhard Veen: While the cost per test is lower for LC-MS/MS compared to immunoassays, the initial investment required for an LC-MS/MS instrument is higher. The use of LC-MS/MS carries several additional financial and logistic considerations not associated with immunoassays, including larger volumes of waste including organic solvents; higher energy costs; greater safety requirements concerning nitrogen tanks and compressors; longer instrument downtime; and a noisier working environment. Automated systems by definition should greatly reduce operating costs, not only through reducing the number of operators required but also through reducing the level of specialized staff training required to achieve the same reliability of results.

What are the economic implications of the 2 approaches?

Gregory Maine: The proliferation of user-defined LC-MS/MS assays and different immunoassays from the various manufacturers, coupled with the lack of assay standardization between these methods, has exacerbated the lack of comparability between TDM methods. This impact is acutely felt, particularly for immunoassays, where attempts to optimize therapeutic drug regimens are confounded by the different test methods employed by the different transplant centers enrolled in multiregimen drug trials. The cost of analysis by TDM pales in comparison to the cost of additional medical interventions, biopsies, etc., as a potential consequence of patients being maintained on less-than-optimal immunosuppressive drug therapies.

Paul Taylor: Automated immunoassays are part of every clinical chemistry laboratory and provide a wide variety of tests, including drug assays. The major costs in performing drug assays on such instrumentation are the reagents. With relatively small sample numbers, this is an ideal approach. For tandem mass spectrometry, the major cost is the instrumentation. Reagent costs are minimal (e.g., our reagent costs for tacrolimus measurement are around $1.50 per test). Thus, tandem mass spectrometry provides a cost-effective approach to measuring large numbers of samples. In this scenario, there must be a tipping point in sample numbers from where initially immunoassays are the most cost-effective and mass spectrometry is ineffective to the reverse situation. Such economic decisions have been made in the area of steroid analysis, where the large pathology providers in the US have switched to LC-MS/MS for these high-sample-number assays (e.g., testosterone and cortisol). Another additional cost for LC-MS/MS is the training and retention of suitably qualified staff to operate this equipment. This may become less of a problem as instrumentation becomes more user-friendly and possibly more automated.
ries should be challenged with external quality controls that are prepared from incurred samples. Corrective action should be mandatory if results are not satisfactory.

The accuracy of either technology is reliant on calibration. For immunoassay users, they are reliant on the manufacturer, but for LC-MS/MS, calibrators prepared in house are typically used. This type of in-house–prepared calibrator has the potential to lead to bias in results. As an example, it has been observed that a heterogeneous group of mass spectrometry assays measuring the same drug and using the same analytical principles produced varied results for the same sample. Recently, there have been some commercially available calibration kits for mass spectrometry methods that may alleviate this potential problem. This may be the way of the future. Finally, stable isotope–labeled internal standards should be obligatory for all LC-MS/MS methods, and confirmatory mass transitions must be employed to ensure data integrity.

**Gerhard Veen:** Instrument and reagent manufacturers must remain committed to continuous further development of TDM assays, reflecting the increasing medical demands and offering state-of-the-art assays that meet the requirements of both the customer and patient.

Current industry status regarding TDM assay standardization is improving, as recent studies under routine conditions have demonstrated across manufacturers. Concurrently, it is important to establish reference methods that are guided and accepted by international associations.

**Pierre Wallemacq:** It is common that different analytical methods yield discrepancies in their results. The strategies for improvement should be based on several approaches, such as (a) using external proficiency-testing schemes to identify a possible calibration bias [free sample enriched with a known amount of drug or a possible interfering compound (metabolite cross-reactivity, patient sample)] and to assess and compare the intramethod imprecision; (b) promoting the use of certified reference materials; (c) encouraging consensus roundtables involving industrial partners, health authorities, and laboratory users to reach international guidelines; (e) using identical certified standards; (e) using the same reported concentration units and specifying the method used; (f) proposing some adjustment in the therapeutic ranges according to the method; (g) developing a reference measurement procedure and implementing a well-accepted validation protocol for in-house LC-MS/MS methods; and (h) retesting with an alternative method in case of suspect results.

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