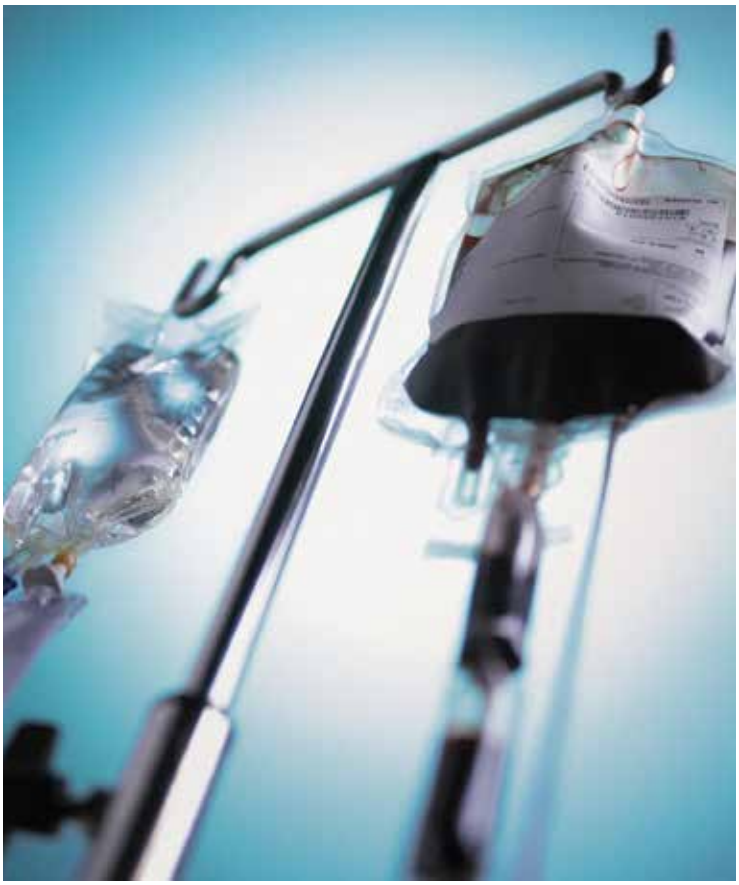
Transforming therapeutic drug monitoring for transplant patients

A Freedom EVO® 150 is being used to full effect by scientists and doctors at the University Hospital of Padova in Italy, preparing samples for LC-MSMS to monitor immunosuppressant drugs in transplant patients.

The University Hospital of Padova is a prominent 1,000-bed hospital in Italy that serves as a specialist center for surgery and liver, heart, kidney and lung transplantation. The Department of Laboratory Medicine in the hospital employs 100 technicians, performing around nine million tests per year. A dedicated emergency laboratory for urgent tests also operates 24 hours a day, carrying out an additional million and a half tests annually. The department's clinical chemistry section has recently overhauled the way it monitors immunosuppressant drugs, choosing LC-MSMS over immunoassays for its better specificity and sensitivity, and its ability to measure several compounds in a single analytical run.



Dr Martina Zaninotto, supervisor of the clinical chemistry section, explained: "Previously, we monitored immunosuppressant drugs using immunoassays but, as assay performance was disappointing, we looked at mass spectrometry as an alternative. Accuracy is of course fundamental to our work and this technique offered us better specificity, to distinguish between drugs and their metabolites, and improved sensitivity for times when we are looking for very low concentrations of drug in a sample."

Dr Mariela Marinova has been closely involved in the method development, in the first instance for determining levels of the immunosuppressants everolimus and sirolimus. She added: "Although mass spectrometry gave us better sensitivity and specificity, manual sample preparation of up to 150 samples a day was proving far too labor intensive. It was clear that we needed to automate this part of the process and, after testing three different systems, we decided that the Tecan workstation offered the best analytical performance for our requirements."

Dr Carlo Artusi, a chemist in the laboratory, described the current set-up: "Preparation of the sample matrix in whole blood is based on protein precipitation, so our Freedom EVO 150 is equipped with Te-Shake™ and TeVacS™ modules to handle 96-well filtration and collection plates. We are currently integrating these stages into our LIS, using the barcode reader on the Freedom EVO to improve sample tracking within the laboratory. Our Agilent 1200 Series LC system coupled to an Agilent 6430 triple quadrupole mass spectrometer with an electrospray ionization interface then runs each sample in just two minutes, creating a very fast, optimized workflow. We are able to analyze our samples far more quickly. Using our previous manual extraction

protocol, sample preparation would take up to two hours, but the Freedom EVO has reduced this to 40 minutes, leaving the technicians free to perform other tasks."

Dr Marinova added: "We are a certified laboratory and time-to-results is an important aspect of this certification; it is vital for patients and clinicians alike to have a quick turnaround of results, and is key for efficient workflows within the laboratory."

Dr Zaninotto concluded: "Automation has really improved the reproducibility of our assays; inconsistencies caused by manual errors have reduced significantly and, in our experience, we haven't needed to repeat a single test since we changed to automated sample preparation. The drug doses given to patients are getting progressively lower, and it is only with the combination of automated sample preparation and mass spectrometry that we can guarantee accurate monitoring of these concentrations. This is especially true for pediatric samples where the drug concentrations are extremely low and, for this reason, we also use this protocol to measure tacrolimus in these patients. We are now developing similar methods for other drugs, including metanephrines in plasma and cortisol in saliva."

The protocol developed by the team at Padova is described in detail in:

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. *Clinical Biochemistry* (2013), **46**, 1723-1727

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For more information on the University Hospital of Padova Department of Laboratory Medicine, visit **www.medlabpd.it**

The clinical chemistry team (left to right): Martina Zaninotto, Mariela Marinova, Prof Mario Plebani, Carlo Artusi, Giorgia Antonelli