

Time saved in diagnostic vitamin B testing

Mass spectrometry is rapidly becoming the method of choice for quantitative analysis of micronutrients such as vitamins B1, B6, B12 and D, as well as for steroids, hormones and many other drugs. For clinical laboratories, the bottleneck of MS-based techniques is not the analysis, but the pre-processing of the samples to remove proteins and other biological molecules that could interfere with the results.

The role of micronutrients in maintaining good health and well-being in an aging population has become increasingly well understood over the past 30 years, but it is only in the last decade or so that reliable assays to effectively detect dietary or metabolic deficiencies of many of these nutrients have become common. This combination of better understanding and more effective testing has seen a huge increase in the number of requests clinical laboratories receive from both primary and secondary care, requiring a change in working practices to accommodate their growing workloads.

Requests for vitamins B1 (thiamine) and B6 (pyridoxine) – which play roles in energy uptake from carbohydrates and fats respectively, as well as being involved in many other metabolic pathways – are among those that have seen a large increase. The Clinical Chemistry Department at Noordwest Ziekenhuisgroep (Northwest Clinics) Alkmaar in the Netherlands now processes over 19,000 assays a year for each of these metabolites alone. Edwin ten Boekel, a Clinical Chemist in the department, explained: “Our department is one of two laboratories that are part of the Northwest Clinics, serving a population of around 600,000 people across the province of North

Holland. It is a very busy laboratory, reporting over 10,000 patient results every day, so we need rapid and efficient analytical processes to ensure we can manage this workload.”

“We originally began quantifying vitamins B1 and B6 over 20 years ago on a HPLC system, however, the assay involves a derivatization step followed by a lengthy analysis time. It also requires a lot of manual pipetting – technicians needed to spend several hours at a time performing very repetitive tasks – and so we recently looked into automating the sample preparation to increase throughput, reduce variability and eliminate pipetting errors. Instead of simply automating our existing protocol, we chose to switch to an ultra high performance liquid chromatography with tandem MS (UHPLC-MSMS) technique, using an automation-friendly protein precipitation step for pre-analytical

sample processing. We investigated several systems to automate this sample preparation, and I was very impressed with the Freedom EVO®

platform following a demonstration in Amsterdam. Our aim was to completely automate the LC-MS sample preparation and so, based on Tecan’s advice, we purchased a Freedom EVO 150 workstation.”

The laboratory’s Freedom EVO was commissioned in the summer of 2016, and has been optimized to work in a 96-well microplate format. Plates containing patients’ hemolyzed whole blood samples, calibrators and quality control samples are placed on the deck of the instrument, and trichloroacetic acid (TCA) is added to each well to precipitate out the plasma proteins. The plate is then sealed and spun down in a centrifuge, and the supernatant from each well is transferred to a fresh 96-well plate using the Tecan system. This analysis-ready plate is then re-sealed and transferred to a standalone UHPLC-MSMS system. Edwin continued: “Although we did not

have any previous experience with Tecan platforms, once you familiarize yourself with the Freedom EVO, the system is very easy to use. The customer support from Tecan has also been good; they

are friendly and we never have to wait long for replies to our queries. We have now implemented several protocols on the instrument, and the reproducibility

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and repeatability of the automated procedure is improved compared to our prior pipetting procedures. We carried out a control experiment by repeatedly processing different batches of the same samples, and obtained a CV of less than five percent, which is well within our acceptable limits. By using a handheld barcode scanner and barcoded microplates, we have also been able

to increase traceability, which is obviously very important in a diagnostics lab. Most importantly, automation of sample preparation on the Freedom EVO has halved the time required for processing the same number of samples. This not only eliminates the need for staff to repetitively pipette samples, it also releases them for other tasks," Edwin concluded.

To find out more on Tecan's clinical solutions, visit diagnostics.tecan.com

For more information on Noordwest Ziekenhuisgroep, go to www.nwz.nl/English/About-us



NWZ's Freedom EVO offers fully automated pre-analytical processing