# Fit for King's

Tecan's AC Extraction Plate™ and Freedom EVO® liquid handling platform have enabled scientists at King's College Hospital to fully automate the analysis of clozapine and norclozapine in plasma, saving time and increasing sample throughput.

King's College Hospital NHS
NHS Foundation Trust

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King's College Hospital, London, is home to a large clinical pathology laboratory offering diagnostic testing services for both the hospital's patients and external samples from across the UK and further afield. Managed by Viapath – a joint venture commercial partnership – the Laboratory has developed a number of automated sample preparation protocols to deal with growing sample numbers and the trend towards high throughput liquid chromatography-mass spectrometry (LC-MS) techniques for a variety of analytical procedures. Lewis Couchman, Senior Clinical Scientist (Clinical Biochemistry Laboratory and Toxicology Department), explained: "One of our most frequently requested assays is for monitoring the drug clozapine – used in the treatment of schizophrenia – and we currently perform about 30,000 tests a year for this drug alone. At present, these samples are prepared manually by liquid-liquid extraction but, with numbers continuing to increase, we wanted to automate the analytical process. The idea was to make it more rapid, more robust and less prone to human error, as well as reducing costs and providing the capacity to grow the service in the future."

"Our department was a test site for the AC Extraction Plate for the analysis of 25-hydroxy-vitamin D in serum and, following this evaluation, I felt this technique could work equally well for our clozapine assay. We carried out some proof-of-concept experiments with excellent results, and approached Tecan. The Company agreed to collaborate with us to further develop the application, and installed a Freedom EVO 100 workstation equipped with an eight-channel Liquid Handling Arm using disposable tips, a Te-Shake™, 16-position sample racks and space to accommodate 96-well sample extract plates, all controlled by Freedom EVOware® software."

Lewis continued: "Initially, we validated the AC Extraction Plate methodology using our existing calibration approach, with analysis of plasma blanks at the beginning and end of every plate, as well as calibration standards and QC material. However, we have now also shown that it generates equally good results using an isotopic internal calibration method. Instead of a batch calibration curve, we add a mixture of isotopically-labeled clozapine and norclozapine standards at three different concentrations covering our calibration range to each sample. This approach gives us an individual, matrix-matched, three-point calibration curve for each sample, and all compounds show excellent recovery. We just need to run QCs to check the validity of the result, and we can generate and report results straight away, without waiting for a batch to finish. It also increases our sample throughput from 65 samples per plate to between 80 and 85 samples, decreasing the cost per test. Automating the AC Extraction Plate procedure on the Freedom EVO makes a real difference to our workflow. Previously, samples arriving at the laboratory by 1 pm would be manually extracted during the afternoon and then run overnight, reporting the results the following day. The Freedom EVO prepares a whole AC Extraction Plate in around 30 minutes, and samples can be run immediately on our LC-MS, with a runtime of just under four minutes a sample."


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Professor Bob Flanagan, Dr Caje Moniz and Lewis Couchman with the Freedom EVO workstation used for automated extraction

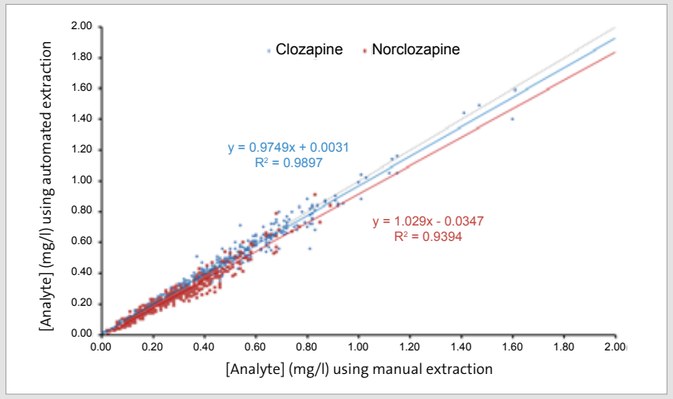
"Controlling the Freedom EVO with Freedom EVOware is very easy; even though the software was new to us, it is really intuitive. With the AC Extraction Plate protocol established, we are fully flexible and can use the same workstation to prepare vitamin D and clozapine samples. There are potentially another 10 to 15 compounds that should work just as nicely using the AC Extraction Plate, including six or seven drugs used in psychiatry that are similar in structure to clozapine. We're also looking at moving our steroid analysis onto the AC Extraction Plate, as the current sample preparation methodology takes a long time to perform."

"Looking forward, we hope to further improve our throughput by evaluating laser diode thermal desorption ionization (LDTD) in conjunction with a plate-based autosampler, eliminating the need for LC and only taking about 10 seconds to analyze each sample. We would then be able to transfer extracts directly from the AC Extraction Plate onto an LDTD plate, and analyze the entire plate in as little as 15 minutes. Our turnaround times would go from days, with manual processing, to just a few hours, which would be fantastic for our therapeutic drug monitoring service!"

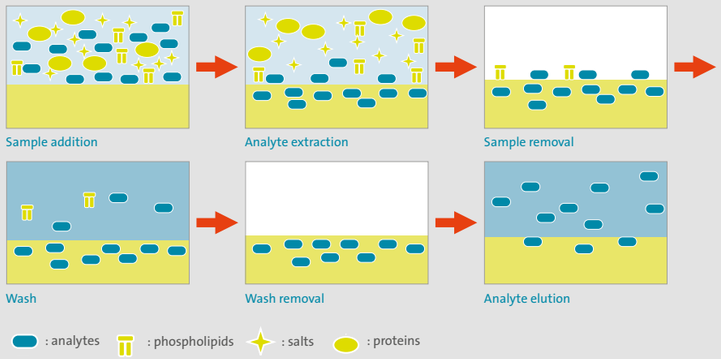
"We've had the Freedom EVO for just a couple of months, but huge amounts of data have already been generated. We have compared around 500 samples prepared manually by liquid-liquid extraction and using the automated method, and are really impressed with the results; there is no discrepancy between the two methods. Currently, we are in the final stages of the evaluation phase, but everybody is excited at the prospect of seeing it implemented," concluded Lewis.

To find out more about Tecan's AC Extraction Plate, visit **www.tecan.com/acplate**

To find out more about Viapath at King's College Hospital, visit **www.viapath.co.uk**




Correlation between manual liquid-liquid extraction (x-axis) and automated extraction using the AC Extraction Plate (y-axis)




The AC Extraction Plate provides a simple extraction protocol