

Automated NGS workflows for greater efficiency in oncology research - A case study from a Center of Excellence in Northern Ireland

David Gonzalez de Castro, PhD FRCPath, and Peter Stewart, PhD, both at Queen's University Belfast, talk about the impact of the AVENIO Edge Liquid Handling System that was installed at Queen's University Belfast early in 2022.

Key takeaways

- Close collaboration between university and clinical research laboratories in Northern Ireland provides evidence for the rapid adoption of NGS in genetic testing.
- The AVENIO Edge—an automated end-to-end liquid handling system for NGS library prep, target enrichment, and normalization and pooling of the libraries—enables NGS sequencing in cancer research labs.
- AVENIO Edge System has proven to be straightforward to adopt for use by non-automation-savvy technicians and scientists.

High ambition and a unique structure

The Center of Excellence at Queen's University is well-positioned to work in parallel with pharmaceutical and biotechnology industries. The Center offers its technologies and research expertise on a collaborative, fee-for-service basis, to accelerate the development and advancement of new tools for high-throughput technologies such as NGS-based genomics, tissue and digital pathology, and big data analytics.

Partners in a parallel new venture

As a key partner to the Center of Excellence, Roche provided laboratory automation, analytical equipment and expertise to the facility at its inception in 2019.

Both Prof. Gonzalez de Castro and his colleague, Prof. Manuel Salto-Tellez, MD-LMS, FRCPath, FRCPI, had previously worked in vendor partnerships with Roche, and Prof. Gonzalez de Castro had also been involved in the independent prelaunch clinical validation of the Roche cobas oncology portfolio.

As a result, Prof. Gonzalez de Castro and Prof. Salto-Tellez were keen to include Roche technology and expertise at the Center of Excellence from the facility's inception. Prof. Gonzalez de Castro explained: "We approached Roche and had an initial meeting at our new facility; we discussed things that we could potentially do together and one of the ideas that was suggested was around integrating genomics and tissue pathology."

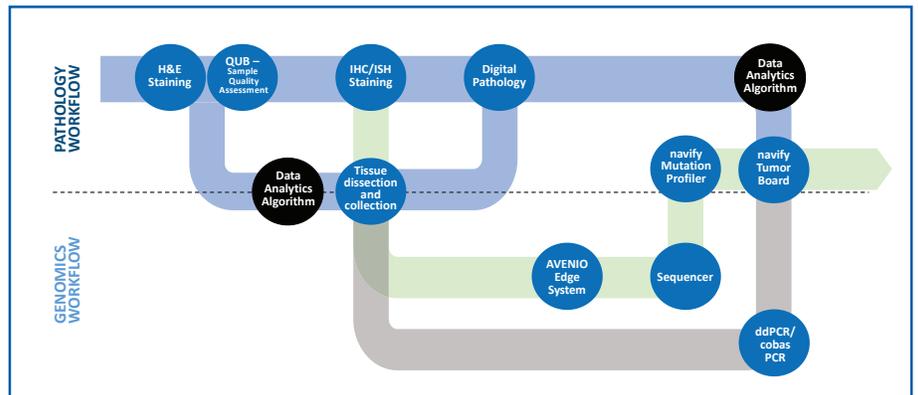


Figure 1: Combined pathology and genomics workflow in the Center for Excellence at Queen's University Belfast.

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He continued: “We have these two elements in the same clinical research lab, which is unique in itself, and this clearly matched the Roche vision of combining digital pathology and genomics in an integrated workflow.”

Given the synergy of the Center's unique expertise and Roche's history as a reputable supplier of equipment and automation in both disciplines, the team realized that they had an ideal opportunity to demonstrate how the integration of disciplines could aid the delivery of better and more actionable genomic results.

The installation and verification of instrumentation with the AVENIO Edge Liquid Handling System was initiated in 2020. However the emergence of the SARS-CoV-2 pandemic inevitably meant that many projects ground to a halt.

Once they were able to install the AVENIO Edge System after the pause caused by the pandemic, Prof. Gonzalez de Castro said: “We basically lost two out of a 4 and half year project... It’s been a major achievement for us to get back on track, and clear the backlog of sample processing, within a year.”

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Advantages and challenges of automation

Liquid handling for sample preparation, NGS library preparation, and pre-analytical research procedures are all areas where molecular pathology, genomics labs, and clinical diagnostics facilities have developed automated solutions to address the challenges of complex, repetitive, resource-intensive workflows.

VANTAGE workflow solution	1x BenchMark ULTRA System
1x VENTANA HE 600 system	1x AVENIO Edge System
2x VENTANA DP 200 slide scanner	

Table 1: Instrumentation provided by Roche for this project.



Automated liquid handling robots can improve method performance, reduce human error, and solve issues of repetitive strain injury (RSI). Importantly, they can also release highly skilled staff to concentrate on more demanding tasks within the laboratory, rather than spending large amounts of time on the mechanics for preparing and running assays. It is generally accepted that NGS pre-analytical processes are particularly demanding when performed manually, with experimental protocols that run over several days and many touchpoints throughout. As a result, many laboratories have invested in 'open-platform' robotic X-Y-Z large-bed systems for NGS library preparation (i.e., hardware that is provided independently from any optimized reagent system and so requires lab users to program the operation of the system to meet their specific lab needs).

When Dr. Stewart, PhD, took up his post as scientific lead at the Center of Excellence at Queen's University Belfast in January 2022, having worked with Gonzalez de Castro's research team since 2016, he was faced with some of the challenges that are often associated with 'open' pre-analytical automation. From experience both within the Queen's University and from discussions with external researchers, it became clear that there are often common and expected challenges with regards to working with automated liquid handling systems. These include limits to the different protocols that the systems can run, reliability issues, and the need for detailed, lengthy staff training to deal with complex programming. Before the AVENIO Edge Liquid Handling System was installed, it had taken the team two years to set up the existing non-Roche and open platform liquid handling system due to the

complex programming that was needed to run their protocols and iron out reproducibility issues. This resulted in a feeling that ‘starting over’ with a new instrument might be something of a ‘poisoned chalice’.

Queen's University Belfast was the first site where the AVENIO Edge System was installed with the goal of automated NGS library prep for cancer sample analysis. While the older non-Roche liquid handling systems were difficult and slow to get set up and programmed, the AVENIO Edge System was set up and running samples with no delays.

Prof. Gonzalez de Castro said: “Based on the team’s experience, there was hesitation, or reluctance, about taking on a new system, particularly given that we had just 12 to 14 months to run through the backlog of samples that had been accumulated due to SARS-CoV-2...”

He continued: “...we knew, of course, that the AVENIO Edge System was different to our existing pre-analytical system—with ready-to-load reagents, validated research protocols, on-board QC capabilities and intuitive workflow management software.”

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Importantly, the team found that in both practice runs and under the pressured real-world research environment, setting up and validating the AVENIO Edge system was straightforward. Dr. Stewart said: “The system was installed during the course of a week, and we were given training over the following week. After that we were able to start running some verification samples.”

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The Center carried out an initial verification run of 24 samples that were sequenced after preparation using the AVENIO Edge System. The results were compared with those from sequencing the same 24 samples from a manual preparation protocol. The comparison was carried out at an external site, Dr. Stewart explained: “We did the comparison across a number of different runs, and when our external collaborators showed us the analysis, we were really pleased to see excellent correlation between results from the manual and the automated preparation.”

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AVENIO Edge System

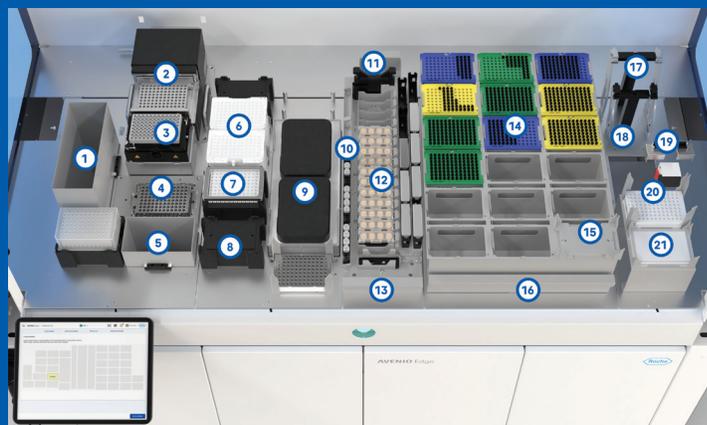
Part of the Roche NGS Automation Solutions, the AVENIO Edge Liquid Handling System—an all-in-one library preparation system—simplifies the inherent complexities of NGS library preparation workflows. The AVENIO Edge accomplishes this by automating key steps and thereby optimizing lab resources, providing users with the freedom to walk away with confidence.

The AVENIO Edge Liquid Handling System minimizes manual touchpoints by including ready-to-load reagents (in the form of barcoded cartridges); validated ready-to-run protocols; on-deck QC capabilities; and intuitive software and user interface. Together, these lead to a reduction of labor-intensive, time-consuming, error-prone, and costly steps of the NGS library prep and target enrichment workflows.

Moreover, the system is 21 CFR Part 11 compliant which makes data integrity and audit trail traceability readily available for the labs that need this regulatory requirement.

The features and benefits of the AVENIO Edge System include:

- Intuitive system software for tracking samples and consumables
- On-deck capabilities that include thermal cycling, library quantification, and dilution and pooling of each sample
- Plug-and-play, barcoded reagents are ready to go from the freezer to the deck before each run, thereby minimizing time spent on filling out source plates or tubes or creating master mixes
- Efficient, consistent, high-quality results
- As little as 20 minutes of hands on time to start a run for a library prep and/or target enrichment workflow
- No specialized liquid handling expertise is required
- 21 CFR Part 11 compliant



1. Tip waste station
2. Thermal cycler
3. Heater/Shaker
4. Proprietary magnetic bead cleanup station
5. Plate and lid waste station
6. Tip park
7. Sample plates
8. Destination PCR plate
9. Cooling unit
10. Tube racks
11. Piercing tool
12. Barcoded cartridge reagents on a rack
13. Reagent drawer
14. P1000, P200, P50 Filtered tips
15. Quantification plate station
16. Consumable drawer
17. Gripper fingers exchange station
18. Quantification module
19. 2D Barcode reader
20. Processing plates
21. Processing plate lids

The AVENIO Edge Liquid Handling System in action

Automated NGS pre-analytical sample preparation using the AVENIO Edge Liquid Handling System has allowed the Center of Excellence at Queen's University Belfast to clear its backlog of pandemic-delayed samples within just 14 months. "There is no way that our Center would have been able to process all the samples using manual prep methods within the project defined timeline." Dr. Stewart added. "The reliability of the AVENIO Edge System has been faultless, and with additional projects being undertaken, the lab can expect an additional 3,000 samples to process for NGS over the course of a year, so using the AVENIO Edge Liquid Handling System frees up staff to be able to either work on different aspects of the same project, or to be able to undertake more projects to bring in additional revenue."

Importantly, the AVENIO Edge uses preoptimized, 'plug-and-play' barcoded reagent kits. Dr. Stewart noted: "You just take (the reagent kits) out of the freezer, defrost, and place them on to the system. This is in contrast to our other liquid handling system, where the first 30-40 minutes of each day is spent making up a master mix." The AVENIO Edge also incorporates multiple sample and consumables checks, together with method and stepwise procedural checks on board. The reagent barcodes are scanned, and expiry dates and lot numbers are checked and recorded as part of the final report. The lab simply selects which protocol is to be run, and operators can either enter the number of samples and other required info, or, more simply, create a CSV file and load that into the system.

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The AVENIO Edge Liquid Handling System also auto-calculates the number of tips, the required reagents, etc. and guides the user on where to place them on the deck. Once the system is loaded with samples, reagents, and consumables, the operator just needs to close the front flap and hit start. The system then carries out a preanalytical check to make sure that the correct tips, reagents, and samples have been loaded. Any discrepancies are automatically flagged, and the run will not start until the problems have been resolved.

"The AVENIO Edge System is the best I've seen around for the ability to track samples and reagents through the lab workflow" Prof. Gonzalez de Castro added.

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In June 2022, in a separate project and in collaboration with another European group, 150 cfDNA samples needed to be sequenced and analyzed, within just two months, to meet the deadline for publication and presentation at a key conference. Dr. Stewart developed a new protocol for the AVENIO Edge System, and the project was completed on time—offering further evidence of how well the system can integrate into a routine sequencing scenario. Dr. Stewart explained: "If we hadn't been able to develop a protocol very quickly on the AVENIO Edge System and get that running, we wouldn't have met the deadline."



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Working with the Center of Excellence to complete the project, the collaborators were able to prepare the poster, which was presented later that year at the 2022 annual conference.¹

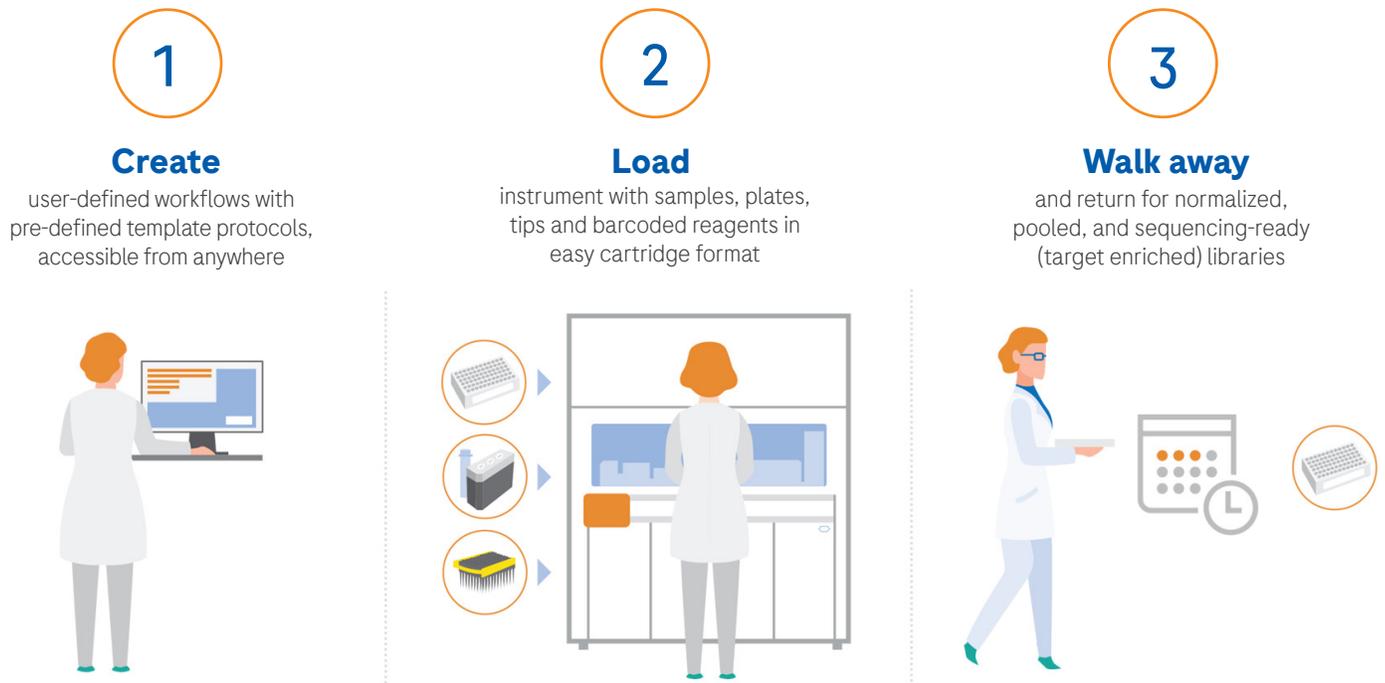


Figure 2: Schematic of method set up and run on AVENIO Edge Liquid Handling System.

Prof. Gonzalez de Castro commented that the Center will now be using the AVENIO Edge Liquid Handling System for other sequencing projects, and that these additional automated protocols are freeing up experienced staff to carry out project-related work rather than spend hours preparing reagents and pipetting. “In fact, much of the manual preparation still carried out for NGS workflows at the Center is designed simply to maintain competency, so there is always a contingency plan if the liquid handlers are off-line” Prof. Gonzalez de Castro noted. Where manual procedures are still being done, the lab has consistently purchased ergonomic pipettes that minimize any risk of RSI, so with both manual and automated systems, the team is still able to pipette safely.

The Center of Excellence at the Queen's University Belfast has since focused on developing NGS panels for different molecular pathology genomic studies.

“We’ve already analyzed three and a half thousand samples from solid tumors per year, which is all the solid tumors that come through the door for genomic testing per year,” Prof. Gonzalez de Castro said.



David Gonzalez de Castro, PhD FRCPath, Co-director of the Center of Excellence and professor of genomic medicine at the Queen's University of Belfast

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Encouragingly, having experienced what the AVENIO Edge System has achieved at the Center, the Department of Medical Genetics at Belfast City Hospital—effectively across the road from the Queen's University Belfast Center for Excellence—is now looking to invest in an AVENIO Edge Liquid Handling System to support the automation of germline analysis in their lab, Prof. Gonzalez de Castro noted. “Hopefully we’ll soon be sharing with them the protocols that Dr. Stewart has validated.”

Conclusion: The AVENIO Edge Liquid Handling System is a solid platform for the future of clinical labs

Importantly, automation enabled by the AVENIO Edge System has played a key role in ensuring NGS-based workflows maintain or improve on overall turnaround times. With the laboratory workflow combining digital pathology, genomics, and data analytics, the lab is able to achieve a three-week turnaround time from sample collection to results. This is ‘huge,’ Prof. Gonzalez de Castro says, when considering that the average turnaround time from samples to data may be 6-8 weeks using more conventional genomics assays.

Dr. Stewart concludes: “AVENIO Edge will play a pivotal role in the future, in order to incorporate NGS-based approaches in clinical labs, to run validated workflows 24/7, with minimal or no manual interventions.”

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Measures of success

With funding from a variety of sources—Northern Ireland regional government, NHS partnerships, Innovation funds and commercial collaborations and contracts, the group in Belfast pays a lot of attention to measuring success against agreed KPIs, and reporting to its stakeholders.

The main funder is Invest Northern Ireland, an institution that provides funding for the growth of the economy in Northern Ireland generally, so it is not healthcare specific. In terms of success, they are looking for economic impact—revenue generated, growth, and how many people are employed as a result of their investment, for example.

The team also measures ‘outputs’. This may be an impact assessment of the translation of services to the healthcare system, or publications, or contract wins. Outputs are important here too, with AI algorithms developed or validated, publications or public engagements, for example, all recorded.



Proven performance in research, and looking ahead to wider applications

In the hands of the team in Belfast, the ‘hands-off’ operation of the AVENIO Edge Liquid Handling System has delivered:

- End-to-end NGS library prep and target enrichment automation
- Reliable, validated workflows
- Simple daily run-time procedures with minimal hands-on time and unattended operation following set up
- Better data security for reporting and audits
- Reduced risk of contamination
- Minimization of error-prone manual processes, from repetitive pipetting to reagent prep and manual data entry
- Controlled protocols that ensure consistent, reproducible results
- Better control of inventory, and significant savings on reagents and consumables
- A reduction in staff-related costs and training time

David Gonzalez de Castro

David obtained his PhD in the field of molecular diagnostics of hematological malignancies in Salamanca, Spain. In 2004, he obtained a position at The Royal Marsden Hospital and The Institute of Cancer Research in London as a Clinical Scientist to lead the Molecular Diagnostics Laboratory. David has been involved in several translational research projects, particularly in the study of predictive and prognostic molecular biomarkers in hematological and solid tumors. He was one of the principal investigators on the CRUK stratified medicine program, working in close collaboration with the pharmaceutical and biotech industry to deliver personalized medicine for cancer patients using genomics. In 2016 he was appointed Professor of Genomic Medicine at Queen’s University Belfast and leads the Genomics and Bioinformatics teams in the Center of Excellence. He also works as a Consultant Clinical Scientist and Lead Scientist for Molecular Diagnostics at the Belfast Health and Social Care Trust. His research is centered around developing and applying genomic stratification tools for precision medicine and to better classify and monitor disease.

Peter Stewart

Peter is the scientific lead for Genomics within the Center of Excellence at the Queen’s University of Belfast. He leads the research and development activities including design, development and validation of genomics technologies and protocols for clinical research in the context of biomarker and clinical studies. This includes optimisation of new automated AVENIO Edge FFPE/HMW genomic DNA and circulating tumour DNA workflows. Peter is a member of the EuroClonality-NGS working group and coordinated the multi-centric validation of the EuroClonality-NGS DNA capture panel as an integrated genomic tool for lymphoproliferative disorders. Prior to working in the Center, Peter has helped support the establishment of a transcriptomic facility as part of an MRC and CRUK funded UK-wide multi-disciplinary stratified medicine consortium, S:CORT. Peter has also undertaken post-doctoral training at the Myeloma Institute for Research and Therapy at the University for Arkansas for Medical Sciences.

Reference

1. Khouja M, Genuardi E, Ferrero S, et al. Genotyping and minimal residual disease (MRD) assessment in cfDNA by the Euroclonality-NGS DNA capture (EC-NDC) panel in mantle cell lymphoma (MCL). *Blood*. 2022;140 (Supplement 1): 3518-3520. doi:10.1182/blood-2022-166043.

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