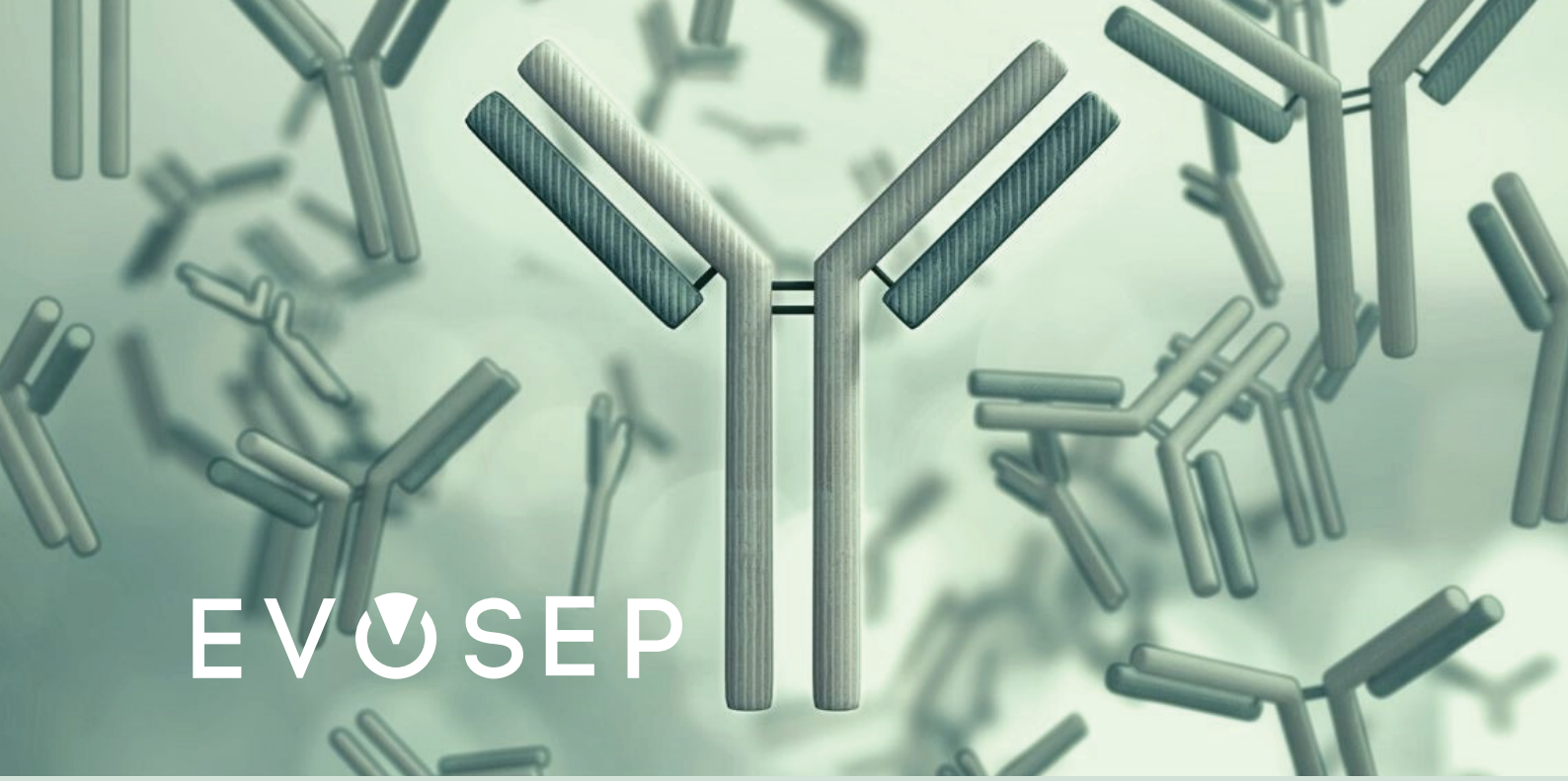


A fleet of Evosep One instruments supports a proteomics factory at Rapid Novor

Introduction

Rapid Novor is a unique business. Based in Kitchener, Ontario (outside Toronto) they have successfully transformed antibody protein sequencing into a streamlined, agile, highly reproducible process. Building on world-class expertise in proteomic analysis, bioinformatics, and big data processing, the company performs fast-turnaround sequencing of any antibody without relying on genetic information. Customers turn to Rapid Novor when they need to identify an antibody in a biological sample like blood, or when they no longer have the expression cell lines of an antibody in their therapeutic pipeline. Their deliverable? A text file with the exact amino acid sequence of the antibody submitted for analysis. Producing high-quality, fully annotated data from biological samples has been a business model for decades. More recently though, rising demand for data has forced analytical service providers to push the envelope in sample throughput and value-adding. Rapid Novor employs Evosep Ones in a standardized setup to eliminate inefficiencies that limit throughput, creating a steady flow of data off eight mass spectrometers.

Paul Taylor, Core Lab Manager at Rapid Novor

"The Evosep One has proven to a high-performing, robust platform for our de novo sequencing workflows. The 30 and 60 samples per day methods and integrated Evotip sample clean-up keep our mass spectrometers running at a very high efficiency rate".

Creating a 'proteomics factory'

Eight mass spectrometers in the core facility at Rapid Novor run 24 hours a day, seven days a week to keep up with growing demand for these analytical services. As the leader of a group of dedicated scientists and technicians, Paul Taylor oversees the smooth and continuous operation of this core

facility. A veteran of mass spectrometry (MS) with 20 years of experience in proteomic analysis for medical and biotechnical industries, Paul was instrumental in scaling up the company's next-generation protein sequencing concept. It was also Paul who introduced a fleet of Evosep Ones as a key node in all the workflows of Rapid Novor. Asked to characterize the fit of Evosep One to the infrastructure of Rapid Novor, Paul says *"it's made for a proteomics factory like ours."*

Adapting mass spectrometry to mass production

'Factory' is no overstatement. *"We're running our spectrometers continuously for weeks on end. We can monitor processes remotely and have outfitted our Evosep Ones with cameras for that purpose,"* Paul explains. Given the number of projects that flow through the core lab, achieving high throughput while offsetting the cost of purchasing, running, and maintaining eight mass spectrometers means reducing as much as possible times when data are not generated. Measures to eliminate manual steps from protocols, reduce maintenance that causes downtime, and prevent unproductive overhead between sample delivery to the mass spectrometers are the building blocks of an efficient, scaled-up, and steady production of data.

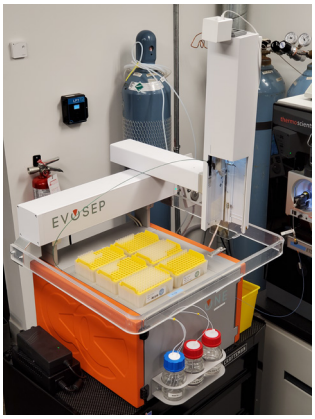
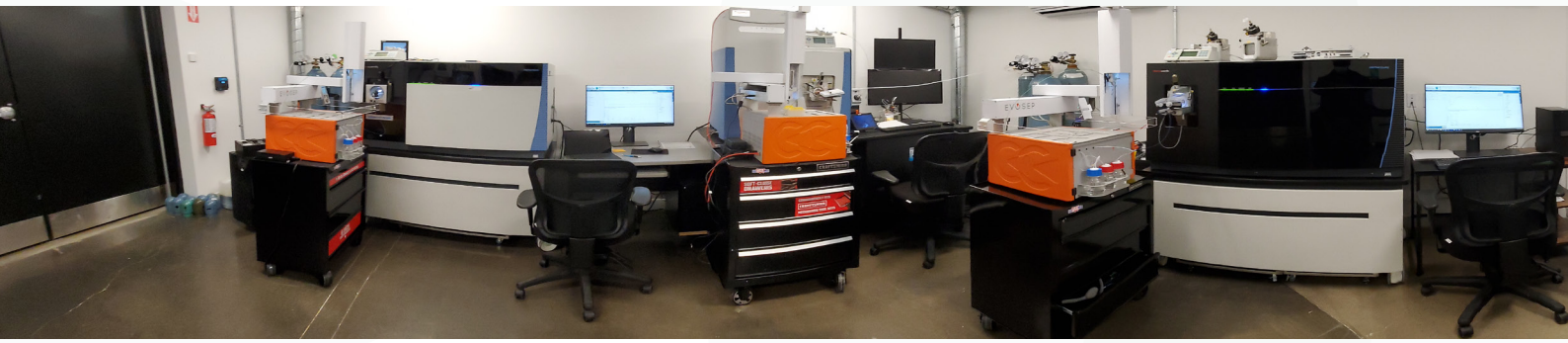


Figure 1: An Evosep One connected to a mass spectrometer at Rapid Novor. To enable continuous data production, the Rapid Novor team uses cameras to remotely monitor each Evosep One as it moves samples through Evotips and onto the liquid chromatography column.

Eight Evosep Ones form a stable liquid chromatography (LC) platform preceding every mass spectrometry measure performed in the Rapid Novor core facility. As such, the fleet of sample separation devices and accompanying Evotips constitute a standardizing point of reference for all workflows, whether part of a service project or an internal experiment. "Regardless of screen," says Paul, "everything eventually goes through an Evotip, and all future analysis protocols currently under development have a definable goal to deliver a sample on an Evotip ready to run on a mass spectrometer."

'The Evotip streamlines and standardizes sample preparation. Samples loaded onto Evotips are desalted and cleared of large impurities prior to injection onto the LC column. In this way, Evotips condense and parallelize multiple preparation steps formerly performed manually on individual samples. Furthermore, each Evotip is a safe, long-term sample storage unit, as peptides in solution in vials bind to surfaces, can be unstable, and some amino acids are susceptible to oxidation. Thus, samples are typically prepared directly before analysis to minimize exposure, but this can lead to deadtime on the analytical column and the mass spectrometer. Paul points out that Rapid Novor has "stored samples on Evotips submerged in liquid for up to one week at room temperature and seen no loss of peptides or evaporation." Paul continues: "This allows us to uncouple sample preparation from instrument scheduling. When samples are due for analysis, we just remove the lid and place the tray of prepared Evotips onto an Evosep One hooked up to a mass spectrometer.



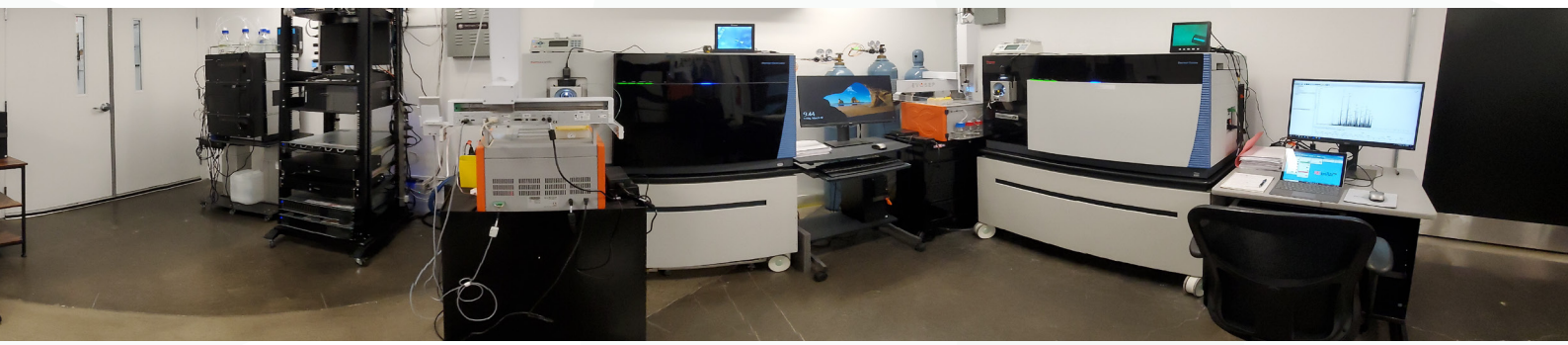
Low pressure pumps on the Evosep One move the eluate from the Evotip into a storage loop while diluting the sample in an offset gradient that pre-separates the peptides before injecting them onto the LC column at a constant, low-flow rate. The offset gradient focuses peaks on the column and controlling the amount of sample entering the column minimizes wear-and-tear, leaks, and technical interruptions. “The Evosep One is designed to be a low-maintenance, robust LC-MS frontend. The Evotip eliminates routine manual work. The Evosep standardized proteomics methods are named according to ‘samples per day.’ Clearly, the team at Evosep understands the concept of a proteomics factory,” concludes Paul.

Goal	Conventional workflows	Workflows with Evosep One
Ensure nanoflow/Cap-flow sensitivty	Change rotors on UHPLC systems	System uses low-flow gradients and has a single high-pressure rotor
Reduce carry-over	Lots of blanks	A new precolumn for every sample
Desalt samples	ZipTips™	Evotips
Store samples	Refrigerated autosamplers	Evotips
Protect analytical column	Off- and in-line filtering, tubing replacement	Evotips
Minimize sample loss	Use low-binding plastics, minimize contact time	Evotips
Boost reproducibility	Use project-specific columns an exact methods	Use project-specific columns and exact methods
Manage instrument efficiency	Variable; need to account for dead volumes, column swaps, number of blanks	Predictable; Evosep standard methods based on samples per day make planning and budgetting more transparent

Table 1: How Evosep One facilitates steady data production in high-throughput LC-MS compared to conventional methods

Plug-and-play standardization

Instrument-to-instrument standardization was and remains a decisive factor for incorporating the Evosep One into all the workflows. “Our mass spectrometers are not permanently outfitted with an Evosep One as a dedicated frontend,” describes Paul. “Instead, each of our eight Evosep Ones sits on a mobile cart and can be connected to any of our mass spectrometers. Switch-over takes less than a minute and we move and position our fleet as needed.” The mass spectrometers in the Rapid Novor core lab use identical method files for the LC component of the workflow and differ only in MS acquisition parameters maintained in a separate file. The upshot is that the technicians can set up runs on any mass spectrometer with the eight Evosep Ones operating indistinguishably, essentially as a harmonized collective frontend LC unit. Paul reveals that he “can’t tell our Evoseps apart. Any variability we see is in the personality of the mass spectrometer.”

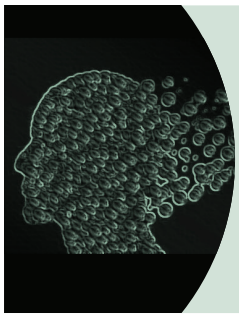


In pursuit of the next level in proteomics

Beyond contracted services, scientists at Rapid Novor push the boundaries of analytical protocols, software capabilities, and applications. Recently awarded funding supported the development of a clinical assay that uses antibody signatures in blood to monitor multiple myeloma in patients and detect relapse earlier. Standardized sample preparation and chromatographic reproducibility is essential to define the parameters of this and future clinical assays, not to mention paramount to achieve meaningful throughput in a clinical setting. Again here, the squad of mobile Evosep Ones are the point of entry where experimental workflows intersect with the tried-and-true methods of the core analytical facility.

Figure 2: A fleet of eight Evosep Ones are shuttled and connected to mass spectrometers that run 24/7, creating a continuous production line of proteomic data. “We were going to name our Evosep Ones after the Seven Dwarfs,” says Paul Taylor, Core Facility Manager. “But now we have eight of them.”

Rapid Novor has also developed a platform to sequence polyclonal antibody mixtures from immunized animals or humans. A critical aspect of this method is the fractionation of samples into component antibodies. Increasingly smaller sample fraction volumes make subsequent analyses impossible, especially when serum amounts are restricted by the body size of the donor – like a mouse. “We are looking at ways to learn more from less sample and I suspect that the nanofluidic capabilities of the Evosep Whisper will play a big part in making that happen,” predicts Paul. Currently, Rapid Novor asks for 100 µg of antibody for analyses but believes that pushing input down to 1 µg would not only eliminate limits in polyclonal antibody sequencing but also make current sequencing services relevant to more steps in pharma development pipelines. “If we could reliably sequence antibodies with five or six proteases on a total sample amount of 1 µg, we would be able to expand our service offerings and generate more revenue.”



Evosep Whisper™ flow Technology

Whisper is the latest Evosep One innovation for ultra-sensitive and stable performance that allow plug-and-play, split-free 100 nl/min performance. The new methods make use of Evosep’s unique low pressure gradient formation and very accurate flow control on the same standard system without technical modifications.

These and other development programs uniquely position Rapid Novor as a trailblazer in decoding immunity directly from biological samples. Combining mass spectrometry and next-generation sequencing data, they already deliver quantified profiles of the complex antibody repertoire in a vial of serum. The Rapid Novor core team’s efficient, modular, high-throughput proteomics factory catalyzes these advances, and eight Evosep Ones are at the core of it all.

EVOSEP

CS-003A 21/04