



Case study

EVUSEP

Rapid Novor: Evosep One delivers high throughput and performance consistency in a clinical proteomics lab

Highlights

- EasyM™ swaps invasive biopsies with accessible serum-based methods for fast and sensitive disease monitoring
- LC-MS based proteomics demonstrates suitability for clinical applications
- Evosep One delivers cost effective, standardized and reproducible results

EasyM – a diagnostic for multiple myeloma unlike any other

Multiple myeloma (MM) is a cancer of plasma cells characterized by the excess production of monoclonal proteins, or M-proteins. Globally, MM is the third most common blood cancer, with an estimated 160,000–170,000 new cases diagnosed each year. Untreated, MM leads to organ damage and failure. Today, many treated patients enter remission, during which minimal residual disease (MRD) is monitored to assess disease status, treatment response, and possible relapse. Traditionally, accurate

MRD detection uses bone marrow biopsies, which are invasive and costly. EasyM offers an alternative by monitoring M protein in serum as a biomarker of MRD, in a fast, sensitive and individualized manner.

“The Evosep One and Evotips eliminate downtime, so we have more time to analyze samples. Thus, we maximize the use of our MS systems, ensuring that the cost of our most expensive instrumentation is not wasted.” Zac estimates that incorporating the Evosep One into the EasyM setup has reduced overhead by at least 80%.

Dr. Zac McDonald,
Senior Scientist & Manager
Proteomics Research at
Rapid Novor



By incorporating the Evosep One into the testing setup of EasyM, Rapid Novor achieves:



80% less overhead



30% shorter LC-MS runtime (30–40 min to 22 min)



Double the throughput



Stable samples at room temperature for 48 hours

Mass spectrometry change the game

Clinical proteomics has long been a focus for Zac. Having ushered its CLIA certification, Zac believes that EasyM demonstrates the suitability of LC-MS for clinical applications. *“Creating antibody-based immunochemistry to target every unique M-protein is impractical,”* he explains. *“De novo sequencing followed by targeted LC-MS quantification is the perfect solution to monitor M-protein, and thus disease, in patients.”* What really motivates Zac is the way EasyM empowers patients. *“We call our assay EasyM because it lets patients assess their disease without painful interventions and uncertainty. I’m excited about getting the power of mass spectrometry to them.”*

Despite advances in LC-MS that have led to throughput, sensitivity, and specificity outstripping alternative methods to identify and quantify proteins in biological specimens, only a handful of LC-MS assays have been approved by regulatory bodies for clinical



purposes. One critical barrier, as Zac points out, is guaranteeing reliable and reproducible results. *“This is a bit of an eye-opener,”* he says. *“In terms of the biology and dealing with a disease, we want to be sensitive. But in the clinical space, higher sensitivity is of little value without reproducibility, accuracy, and quality assurance.”*

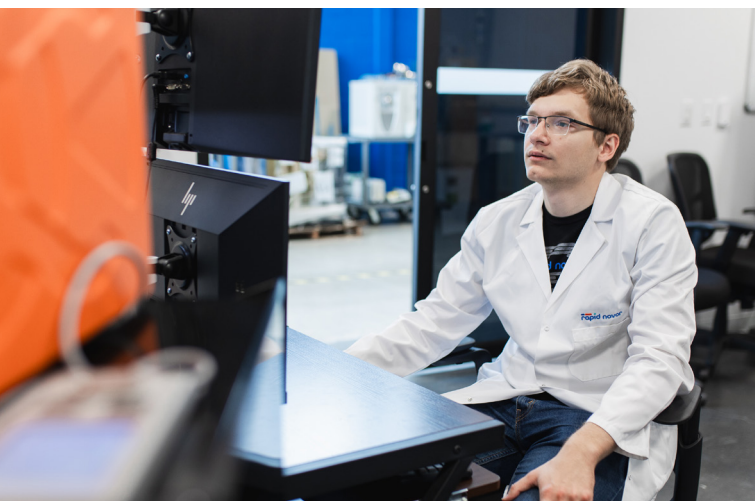
Evosep One streamlines sample separation

The initial proof of concept for EasyM was published in 2021¹ after which the Evosep One was incorporated into the workflow for sample separation to increase throughput. Thanks to the Evosep One, the EasyM testing personnel at Rapid Novor perform 60 runs per day for 1 to 2 weeks without intervention – twice as much as with alternative liquid chromatography (LC) systems. The disposable Evotips minimize carryover without the lengthy washing and preparation of trap columns in those systems.

“Minimal retention time variation is important for reproducible results with our EasyM assay. The Evosep One has shown consistent performance both across instruments and over time, and samples remain stable for 48 hours in Evotips at room temperature.”

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"In our experience, washing trap columns leads to considerable downtime," explains Zac. "We had to disconnect capillaries, remove blockages, and repair the system. With the Evosep One, we can load a lot of Evotips at once and run long sequences without reloading or cleaning, which really reduces downtime. In fact, we could not get through large numbers of samples in a timely manner without the Evosep system."

The reduced downtime of the Evosep One also translates into decreased overhead, which saves costs. *"When you're washing a trap column, you're not running anything on your mass spec. That's overhead. The Evosep One and Evotips eliminate that downtime, so we have more time to analyze samples. Thus, we maximize the use of our MS systems, ensuring that the cost of our most expensive instrumentation is not wasted."* Zac estimates that incorporating the Evosep One into the EasyM setup has reduced overhead by at least 80%.

"In the clinical space, robustness is paramount"

Comparing the needs of a clinical to a research proteomics lab, Zac highlights notable differences: *"For research labs, pushing the limits of sensitivity is a strong driver. In the clinical lab, however, we strive for standardization and reproducibility to deliver reliable results. In the clinical space, robustness is paramount."* The Evosep One and Evotips not only facilitate cost-effective processing and throughput but also enhance the robustness of the EasyM assay in three key ways:

1. Evotips stabilize samples: Internal

validation studies at Rapid Novor demonstrate that samples kept on Evotips for EasyM analysis show few modifications and almost no peptide loss. This contrasts with other LC systems where samples are held cold in vials. *"We would get peptide loss to the surface of the vials, which limited the time we could allow samples to sit,"* says Zac. *"In Evotips, samples remain stable on the instrument for 48 hours at room temperature."*

2. Evosep One supports instrument-to-instrument consistency: As a monitoring assay, the EasyM must generate comparable results regardless of the instrument used to sequence and quantify M-protein. *"Each instrument pair – mass spec and Evosep – is validated back to a central instrument that operates within narrow tolerances. So, if we run a sample on two different instruments, they must give us the same results. Instrument-to-instrument consistency requirements are particularly high for EasyM, and the Evosep system shows that consistency."*

3. Evosep One offers standardized gradients: Evosep One is designed to minimize operator variability. *"Another advantage of Evosep is that our technicians learn to run the EasyM protocol without too much effort,"* says Zac. Six ready-to-use separation gradients make using the Evosep One intuitive. The EasyM uses a lengthy gradient for sequencing, where high separation power is essential, and a shorter gradient for quantification, where target peptides in a defined retention time



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window are quantified. Zac argues that the *“standardized runs on the Evosep are very attractive in the clinic. Operators can use the system quite easily, and there isn’t much room to unintentionally change the operation of the instrument.”*

Standardization and automation is paving the way for clinical proteomics

Reproducibility was an important criterion for EasyM’s CLIA certification. *“Our validations focused on getting our systems to a point where we can analyze a patient sample today, in six months, and then in a year and know that we’re getting comparable results. We worked with Evosep engineers to attain the reliable retention times we needed so that our mass specs and Evoseps now operate within tight margins.”* Zac added that the stringent requirements

About Rapid Novor

Rapid Novor enables reliable discovery and development of novel reagents, diagnostics, and therapeutics.

Thanks to their Next Generation Protein Sequencing and antibody discovery services, researchers have furthered thousands of projects, patented antibody therapeutics, and developed the first recombinant polyclonal antibody diagnostics. The company performs fast-turnaround sequencing of any antibody without relying on genetic information.



for EasyM were uncommon, but Evosep engineers provided the necessary support and documentation to qualify the installation and operation of each system running in the testing lab.

Zac and his team are motivated to keep moving. *“We want to reduce the complexity of EasyM. We’re looking to automate the loading of Evotips, which is amenable to robotics, and reduce our runtime even further.”* As Zac puts it: *“We need to standardize. We need systems that run without intervention for long periods of time. We need reference materials to confirm comparable outcomes between labs. We need to speak the regulators’ language and set up our labs that way. That is when the dream of clinical proteomics becomes reality.”*

- 1 McDonald, Z. et al. 2021. Mass spectrometry provides a highly sensitive, noninvasive means of sequencing and tracking M-protein in the blood of multiple myeloma patients. Journal of Proteome Research 20: 4176. 10.1021/acs.jproteome.0c01022



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