

Field-testing the Evosep One at the Max-Planck-Institute for Biochemistry: PAVING THE WAY TO CLINICAL PROTEOMICS

A substantial research effort at Matthias Mann's group at the Max-Planck Institute of Biochemistry in Martinsried near Munich is dedicated to improve patient care by bringing proteomics to the clinic. Liquid chromatography-mass spectrometry (LC-MS) methods are core to their approach, and a fast and robust performance of the LC-MS workflow is therefore an absolute prerequisite for successful clinical proteomics.

During a 6-months' field-test of the Evosep One instrument, scientists in Prof. Mann's laboratory were able to significantly increase robustness in liquid chromatography and to reduce LC run time to just 24 minutes. Implementing the Evosep One—a 'gradient off-set focusing HPLC instrument'—into their workflow removed a major hurdle in the process of establishing proteomics as a standard diagnostic method.

The goal: Bringing proteomics into the clinics

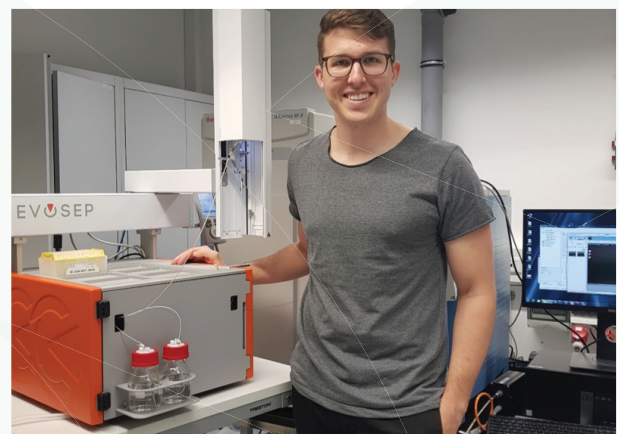
Biomarker tests have become an important tool in clinical diagnostics to analyze plasma, urine, saliva, or cerebrospinal fluid. The standard method for biomarker detection in patient care is to use antibody-based assays with plasma samples. While these immunoassays address the throughput needed in the clinic, their limited specificity restricts biomarker analysis to highly abundant proteins. A workflow based on mass-spectrometry could lower the detection threshold and unlock the full potential of biomarkers. This method, however, currently lacks the speed and robustness needed for clinical use.

The goal of the team in Martinsried is therefore to establish a fast and streamlined workflow for clinical proteomics. First, they implemented a fully automated workflow that allows to prepare 96 samples in parallel, and that delivers a purified, ready-to-analyze peptide mix within two hours. Subsequently, the scientists reduced the time needed for LC column separation from two hours to just 20 minutes. Still, the LC instrument required 15 minutes of overhead time for each measurement,

which nearly halves the possible throughput.

But an even larger problem for the team was the frequent instrument downtime. As Philipp Geyer explains: "About 80% of our measurement errors were due to leaks or similar issues in the HPLC instrument." With all other steps of the workflow streamlined and automated, this instability in liquid chromatography presented a major hurdle on the way to implementation in the clinic.

Introducing the Evosep One



Evosep supports the implementation of proteomics into the clinic by making sample separation 10 times faster and 100 times more robust than today's alternatives. The Evosep One achieves these goals by introducing a set of novel features.

- Instead of injecting the liquid sample from a vial, the instrument elutes the sample directly from the desalting tip using a gradient created by two low-pressure pumps. Using a disposable tip greatly reduces cross-contamination. At the same time, the tip acts as a pre-column to remove impurities that might otherwise affect LC column performance.

- A second set of low-pressure pumps creates a gradient offset that dilutes the tip eluate on its way into a storage loop.
- A high-pressure pump injects the gradient with the pre-separated sample from the storage loop into the LC column. Using only one high-pressure pump makes the instrument a lot more robust, reducing wear and tear.
- Because of the offset gradient, sample molecules are focused very efficiently and therefore more efficiently detected by the mass spectrometer.

Field-test at the MPI Martinsried

As the first scientist to join Prof. Mann's initiative on clinical proteomics in 2014, Philipp was the ideal candidate to field-test the Evosep One. In a series of

experiments using HeLa cells, he showed that with the Evosep One, the cross contamination rate was improved 10 fold over current state-of-the-art methods. (Figure 1).

With only three minutes of overhead, the overall analysis time was shortened from 35 to just 24 minutes. But to Philipp, the observed increase in robustness is even more important. "Having only one high-pressure pump instead of three, and the very simple way of loading the instrument, makes the Evosep One a lot more robust than other instruments," he states. "During the field-test, we analyzed more than 2,000 samples in one study. With the last version of the instrument, we did not have any issues at all so far." (Figure 2). To further validate his results, Philipp is now going to repeat an already published study that analyzes the effect of proteomics upon weight loss with a set of 320 samples.

Figure 1: With the Evosep One, cross contamination rates of 0.035% are obtained. Top: Experimental setup of HeLa cell samples and blanks. Bottom left: MS spectra of samples 1-4. Bottom right: Determination of cross contamination rate.

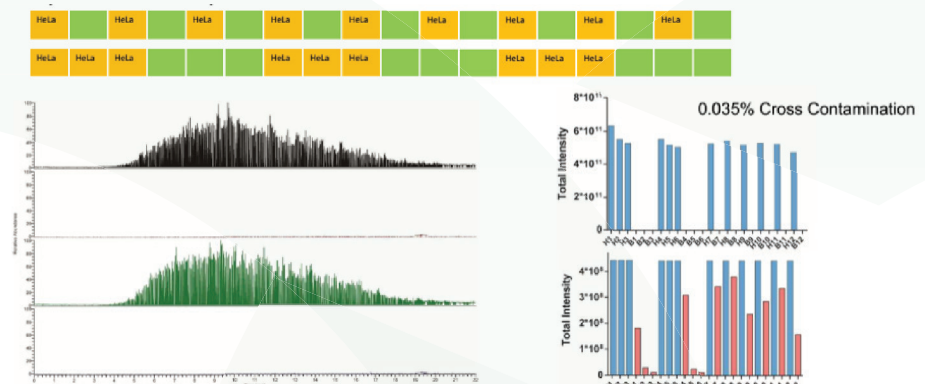
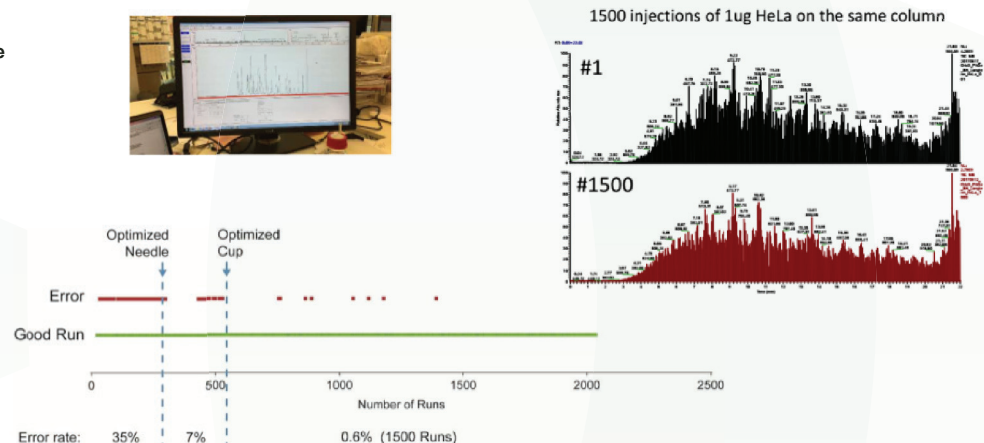


Figure 2: During the Evosep One field-test, error rates could be reduced to 0.6%, running 1500 injections on the same column.



“It was very exciting to follow the journey of the Evosep One first-hand, and to help optimize the instrument to an even more robust and reliable system. As a user, it was fun to communicate our ideas on new features and adjustments, such as fine-tuning the programmed gradients,” Philipp says. “Interacting with the Evosep team was a great experience, because they were very responsive to our feedback and always provided fast, friendly, and competent support.”

Conclusion

With the Evosep One, the proteomics experts in Martinsried were able to significantly increase robustness and speed of liquid chromatography within just a few months' optimization time, thereby removing a major hurdle on their way of bringing proteomics to the clinics. While shorter overhead reduced the time to complete a run to only 24 minutes, the increased robustness of the instrument saved significant time previously spent on fixing errors. More importantly, the Evosep One provided enhanced measurement reproducibility and minimized cross-contamination, two main prerequisites when working with precious patient samples.

As Philipp concludes:

„With mass spectrometry instruments getting smaller and easier to use, I believe that the Evosep One can really be transformative for the process of bringing proteomics into the clinic, eventually enabling a fast, fully automated process from sample to result”.

References

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