
**Dr. Philipp Geyer and the Evosep One in the Max-Planck-Institute laboratory**

## Evosep One Field Test at the Max-Planck-Institute of Biochemistry:

# PAVING THE WAY TO CLINICAL PROTEOMICS

Research efforts of Matthias Mann's group at the Max-Planck Institute of Biochemistry in Martinsried near Munich aim to improve patient care by bringing proteomics to the clinic (1,2). Liquid chromatography-mass spectrometry (LC-MS) methods are core to their approach. Fast and robust performance of the LC-MS workflow is therefore an absolute prerequisite for successful clinical proteomics.

During a 6-month field test of the Evosep One instrument, scientists in Prof. Mann's laboratory significantly increased the robustness of their liquid chromatography methods and reduced their 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 to establishing proteomics as a standard diagnostic method.

## The goal: Bringing proteomics to the clinic

Antibody-based protein biomarker tests still remain the standard tools in clinical diagnostics to analyze plasma and other body fluids such as urine, saliva, or cerebrospinal fluid. While these immunoassays address the throughput needed in the clinic, there are concerns about their limited specificity and incompatibility with multiplexing. Mass spectrometry (MS)-based proteomics has the potential to unlock the full potential of biomarker assays, as it provides enhanced sensitivity and lower detection thresholds. MS, however, currently lacks the speed and robustness needed for use in patient care.

In order to bring proteomics to the clinic, the main goal of the Martinsried team is to establish a fast and streamlined LC-MS workflow. First,

they implemented a fully-automated, two-hour procedure for simultaneous preparation of 96 samples that delivers purified, ready-to-analyze peptide mixtures. 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 (e.g., sample loading and column equilibration) for each measurement. This extra time significantly reduced MS productivity and nearly halved the possible throughput.

But an even larger problem for the team was the frequent LC system downtime. As Dr. Philipp Geyer, scientist in Prof. Mann's team, explains, "About 80% of our measurement issues were due to leaks or other problems with the LC instrument." With the other steps of the workflow largely streamlined and automated, this instability in the liquid chromatography step presented a major hurdle on the way to implementation in the clinic.

## Introducing the Evosep One

Evosep facilitates adoption of proteomics in the clinic by making sample separation 10 times faster and 100 times more robust than today's alternatives. The Evosep One instrument achieves these new standards with a set of novel features:

- Instead of injecting the liquid sample from a vial, it is eluted from the desalting tip with a gradient created by two low-pressure pumps. Use of 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 while it is transported into a storage loop. From the storage loop, a high-pressure pump injects the gradient together with the pre-separated sample into the LC column. Use of only one high-pressure pump, operating at a constant flowrate, makes the instrument extremely robust, and reduces wear and tear.
- Because of the offset gradient, sample molecules are more efficiently focused, providing uncompromised separation and increased sensitivity in the subsequent mass spectrometric detection.

## Field test at the MPI Martinsried

As the lead scientist for Prof. Mann's initiative on clinical proteomics, Dr. Philipp Geyer was the ideal candidate to field-test the Evosep One. In a series of experiments using HeLa cells, he showed that cross-contamination rate was reduced to extremely small values *(Figure 1)*.

With the achievement of negligible overhead time, LC separation was significantly faster, and sample throughput nearly doubled. 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. In the last set of 1,500 samples, we observed only a few errors with the prototype instrument that were attributed to tips not correctly loaded with sample (Figure 2)."* To further validate his results, Philipp is now repeating an already published study with a set of 320 samples that investigated the effect of weight loss on plasma proteomics (2). By employing a combination of LC on the Evosep One with BoxCar, a new MS scan mode, he expects to be able to reduce overall measurement time to 20% of its original value, while still maintaining excellent result quality.

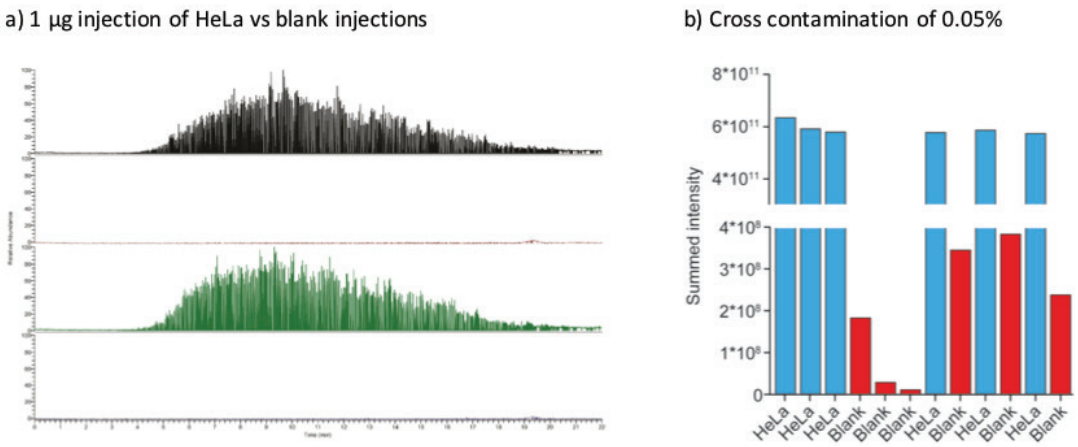


*Figure 1:* **The Evosep One keeps cross-contamination to a minimum.** a) MS spectra of Hela – blank – HeLa – blank. b) The experimentally determined, average cross-contamination rate was 0.05%.

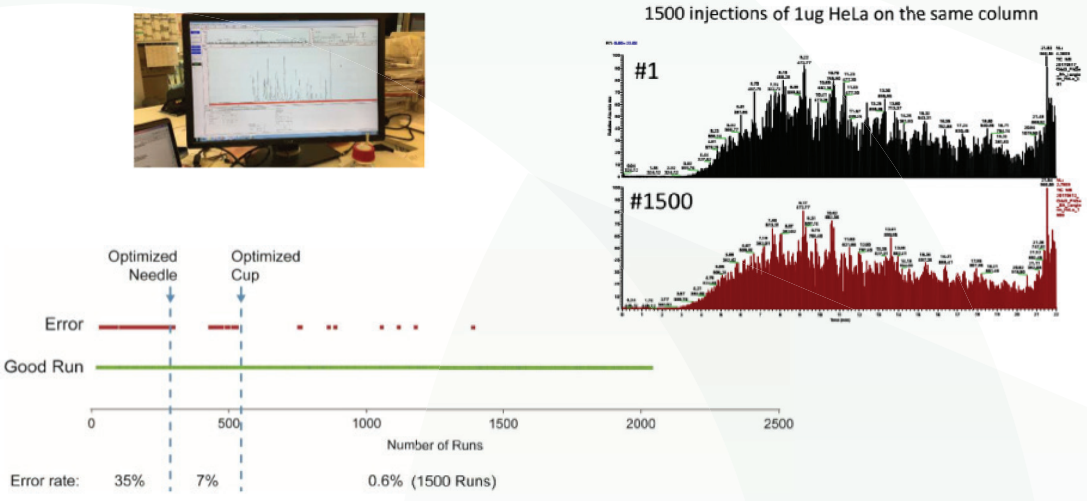


*Figure 2:* **During initial Evosep One field tests, error rates were reduced to 0.6%, running 1500 injections on the same column. The only issues that were observed did not trace back to instrument performance, but to loading of the tips.**

***"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 significantly increased robustness and speed of liquid chromatography within just a few months, thereby removing a major hurdle on their way to bring proteomics to the clinic. In addition to reducing the time to complete a run to only 24 minutes, the increased robustness of the instrument saved hours previously spent on fixing errors. More importantly, the Evosep One enhanced measurement reproducibility and minimized cross-contamination, two top requirements 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."***

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**References**


1. Geyer, P.E., Kulak, N.A., Pichler, G., Holdt, L.M., Teupser, D., Mann, M. (2016) Plasma Proteome Profiling to Assess Human Health and Disease. Cell Systems 2, 185-195. *http://pubman.mpdl.mpg.de/pubman/item/escidoc:2352959:3/component/escidoc:2352964/1-s2.0-S2405471216300722-main.pdf*.
2. Geyer, P.E., Wewer Albrechtsen, N.J., Tyanova, S., Grassl, N., Iepsen, E.W., Lundgren J., Madsbad, S., Holst, J.J., Torekov, S.S., Mann, M. (2016) Proteomics reveals the effects of sustained weight loss on the human plasma proteome. Molecular Systems Biology 12: 901. *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5199119/pdf/MSB-12-901.pdf*


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