Automated Evotip®-assisted Sample Clean-up for LC-MS using Opentrons Flex®

Written by

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INTRODUCTION

Desalting using disposable tips has become increasingly popular in sample preparation for LC-MS. Evotips, combined with Evosep® One technology, simplify and standardize the sample loading process to enhance the throughput of analysis. Digested proteins are desalted and stored in Evotips, then fed into the LC-MS instrument through the Evosep One system.

The automated sample loading workflow can be automated on the Opentrons Flex liquid handler, using a layered sandwich approach (Figure 1). The pipette dispenses the liquid through the Evotips, performing equilibration, target capture, and wash steps and leaving 100-120 µL of solvent A in the Evotips to ensure secure sample storage. These Evotips are ready for injection into the Evosep One. This on-robot, end-to-end sample processing eliminates the need for manual intervention.

Here we present three independent and comprehensive experiments utilizing HeLa protein digest and human plasma samples, to rigorously demonstrate the flexibility, robustness, and reliability of this automated method for seamless, end-to-end sample preparation with the Opentrons Flex.

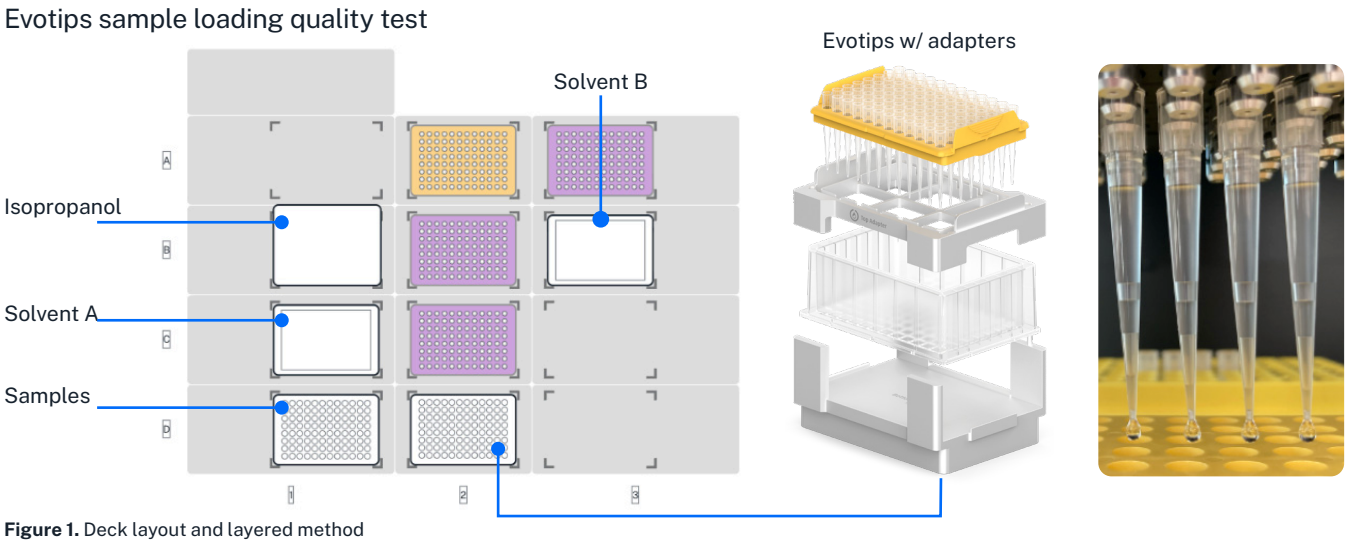

Figure 1. Deck layout and layered method

METHODS

Three complementary workflows were used to prepare samples and perform LC–MS analysis. **In Method 1**, a HeLa protein digest (50 ng in 20 µL solvent A) was prepared and loaded onto Evotips either manually or using an Opentrons Flex automation platform. Samples were analyzed by LC–MS on a Bruker timsTOF Pro 2 using a 60 samples-per-day (SPD) method, and data were processed in Spectronaut using the directDIA workflow.

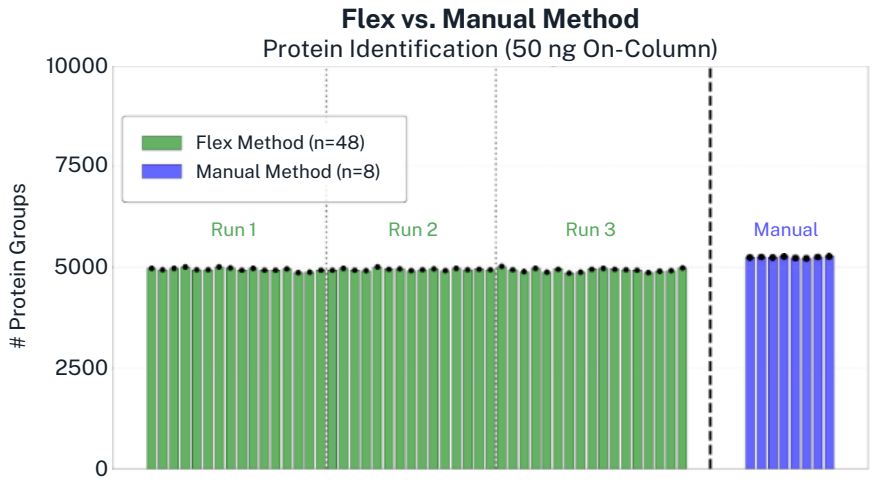

Figure 2. Across 48 samples, data confirm the consistency of the Flex protocol and its comparability to 48 manually processed samples

In Method 2, Evotips were prepared and loaded manually using centrifugation or on the Opentrons Flex platform. Tips were sequentially cleaned with isopropanol, rinsed with Solvent B (0.1% formic acid in acetonitrile), and equilibrated with Solvent A (0.1% formic acid in water). MS-Compatible Human Protein Extract Digest (50 ng in 20 µL Solvent A, human K562 cell digest, Promega Corporation) was loaded onto the tips followed by a Solvent A wash. The loaded tips were stored submerged in LC/MS-grade water until ready for analysis. LC–MS analysis was performed on a timsTOF Ultra 2 coupled to an Evosep LC system via a CaptiveSpray 2 source. Peptides were separated on a C18 IonOpticks column using the Evosep Whisper Zoom 40 SPD method and analyzed by dia-PASEF with defined m/z and ion mobility ranges.

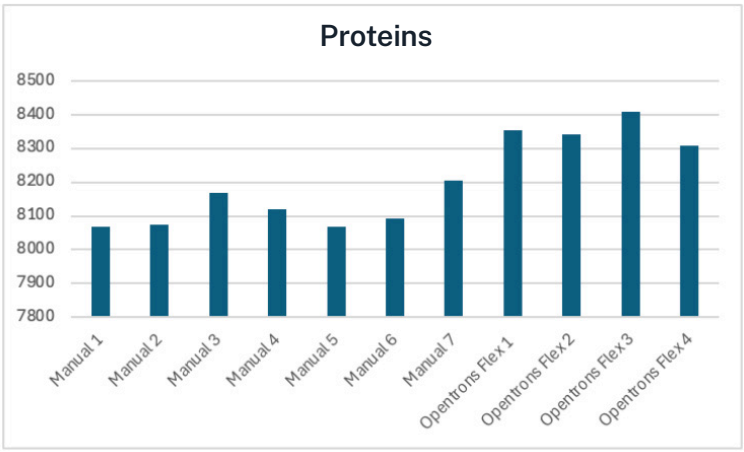

Figure 3. Improved protein identification automated methods on the Opentrons Flex

In Method 3, plasma samples (5 µL per replicate, n = 8) were depleted using a modified PCAn workflow on the Opentrons Flex platform. Proteins were denatured with CAA/TCEP and digested on SP3 beads using automated handling, and resulting peptides were loaded onto Evotips on the Flex. LC–MS analysis was performed using an 88-minute Evosep separation (15 SPD method) coupled to a Thermo Orbitrap HF-X mass spectrometer operating in DIA mode. Raw MS data were analyzed in Spectronaut using the directDIA workflow.

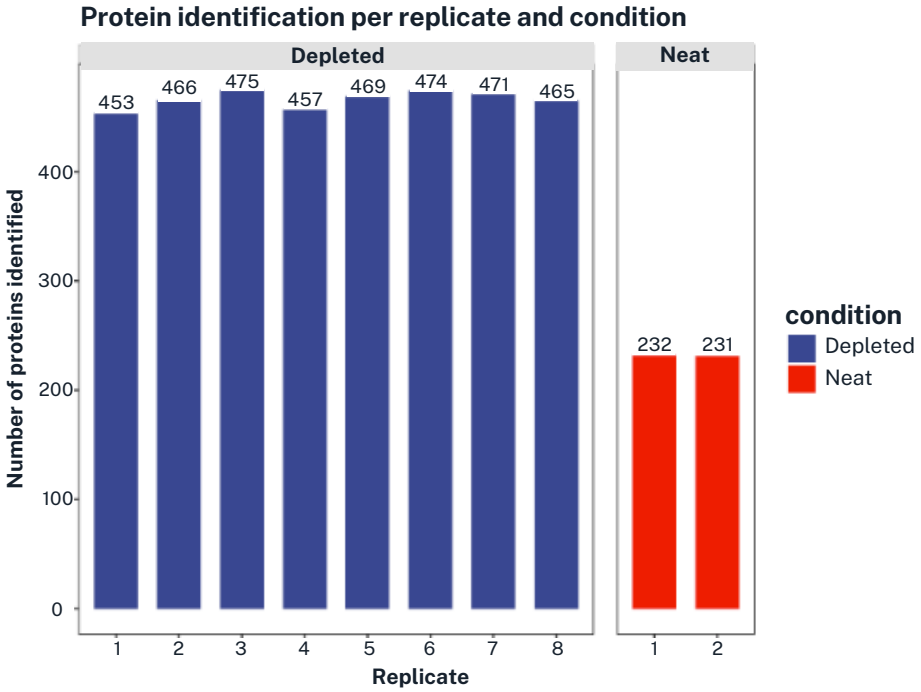

Figure 4. A two-fold increase in protein IDs was observed after enrichment (depletion)

RESULTS

The data from 3 runs of the Flex protocol, each processing 16 Evotips (2 columns), confirm the reproducibility between runs. Across 48 HeLa samples, data confirm the consistency of the Flex protocol and its comparability to 48 manually processed samples (Figure 2). When preparing MS-Compatible Human Protein Extract Digests, the automated preparation yielded more proteins identified than the manual control experiments (Figure 3). A two-fold increase in protein IDs was observed after enrichment of plasma samples, and the results also confirmed the consistency of Flex protocol across all Evotips processed (Figure 4). The Flex protocol demonstrated strong run-to-run reproducibility, as confirmed by data from three runs, each processing 16 Evotips (two columns). Across 48 HeLa samples, the consistency of the automated Flex protocol was established, showing comparability to 48 manually processed samples (Figure 2). Notably, the automated preparation of MS-Compatible Human Protein Extract Digests resulted in a higher number of protein identifications compared to the manual control experiments (Figure 3). Furthermore, a two-fold increase in protein IDs was observed following the enrichment of plasma samples (Figure 4).

CONCLUSION

To rigorously validate the performance of our automated sample preparation solution, we executed three exhaustive and entirely independent experimental campaigns. These studies were strategically designed to encompass diverse and challenging sample matrices, ensuring a comprehensive assessment of the method's capabilities. Specifically, we employed a well-characterized HeLa protein digest, serving as a high-quality, reproducible standard, alongside complex human plasma, a highly relevant and challenging biological matrix frequently encountered in clinical and proteomic research.

The integration of these varied matrices confirms the system's broad applicability and superior performance across distinct sample types, a critical requirement for modern, high-throughput biological laboratories.