Sweat Proteomics: A Noninvasive Approach for Biomarker Discovery with Optimized and Fully Automated Sample Preparation

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Abstract: Sweat, recently considered a simple biofluid mainly composed of water and electrolytes, has been found to contain a diverse range of proteins and metabolites that can serve as biomarkers for clinical diagnosis. Unlike serum or plasma, sweat collection is noninvasive, making it an attractive alternative for biomedical research and therapeutic monitoring. Mass spectrometry (MS)-based proteomics enables identification and quantification of sweat proteins, which is crucial for biomarker discovery. In our previous study, we have identified over 1000 unique proteins in sweat, many linked to biologically significant functions, confirming thus their potential as disease biomarkers.

A major challenge in proteomic analysis is sample preparation, as it impacts detection sensitivity and reproducibility. The conventional precipitation methods cause protein loss, reducing overall sensitivity and impact reproducibility. To improve both protein recovery and reproducibility, we have investigated an alternative method: the "One Pot" method, which processes sweat samples directly and compared it with an ACN precipitation method and the use of "2D Clean Up" kit, a commercial protein purification kit. Each method was tested in six replicates (total of 18 samples, 50 ng of sweat proteins each). The study identified 2144 unique proteins across all methods, with 1020 proteins common among them. The One Pot method recovered 173 exclusive proteins, while the 2D Clean Up kit identified 95 unique proteins, and ACN precipitation only 13, showing the lowest recovery. Additionally, the One Pot method exhibited 1.4x and 1.3x higher signal intensity compared to ACN precipitation and the 2D Clean Up kit, respectively.

These findings confirm that the One Pot approach provides higher sensitivity, better qualitative and quantitative results, and improved protein recovery. Notably, this method requires only 4 µL of sweat, with an analysis conducted on only 1/20th of this initial volume, ensuring minimal sample consumption while maintaining robust detection capabilities. Morover, its compatibility with automated high-throughput analysis makes it ideal for large-scale studies. When paired with the Mantis® robot, the One Pot method allowed for simultaneous preparation of 152 sweat samples, significantly enhancing efficiency in proteomic workflows.