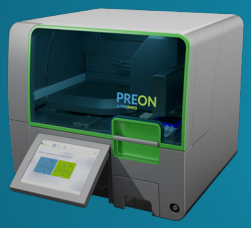
PreON coupled to the iST-BCT kit: A fully automated and reproducible proteomic workflow for biological fluids



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Introduction

Biological fluids (Biofluids) such as plasma, serum, and CSF are enriched with secreted proteins and metabolites. The biofluid proteome reflects changes in disease progressions and is sensitive to changes in the environment, lifestyle, dietary habits, or drug treatments.[1,2] They can provide important information at a systemic level, especially through their involvement in transporting biological molecules. For these reasons, they are often collected and banked for future clinical studies. Blood, in the form of plasma and serum, is a biofluid of choice because it can be collected with a minimally invasive procedure and is rich in biological information about the whole organism. Cerebrospinal fluid (CSF) is beneficial for assessing the state of the brain or the central nervous system.

High-throughput proteomic studies of biofluids from large cohorts of patients/animal models require rapid, robust, and reproducible sample preparation. Manual sample preparation is prone to error and day-to-day variability. An effective solution is to automate well-established protocols, thereby improving the results compared to manual handling over long periods of time while freeing up human time for other crucial tasks.

In this work, we evaluated the efficiency and reproducibility of the iST-BCT kit on a PreON automation platform. Diverse types of biofluids were selected to illustrate the versatility of the iST-BCT kit. Experimental replication using the PreON platform was performed over a period of 9 days to demonstrate the long-term repeatability of the automated proteomics workflow.

Keywords

Automation, PreON, Biofluids, Sample preparation, Protein identification, Clinical proteomics, Biomarker discovery, Liquid biopsies

Key takeaways

PreON eliminates the hands-on time and enables fully automated processing of samples for mass spectrometry-based protein analysis.

The iST-BCT workflow coupled with PreON allows straightforward analysis of various types of biological fluids, such as plasma, serum, and CSF.

Standardized and highly reproducible sample preparation workflow for reliable proteomic profiling of a wide range of biofluids.

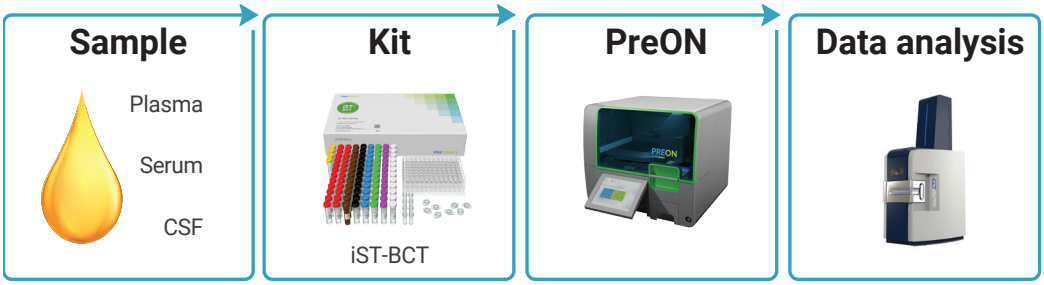

Figure 1 | A fully automated sample preparation for proteomics workflow of biofluids analysis using the PreON platform coupled with the iST-BCT kit.

Methods

A human plasma sample, a BALB/C mouse plasma pool sample, and Sprague Dawley rat plasma, serum, and CSF pool samples were obtained from BioIVT (West Sussex, UK). All samples were collected from male donors, and plasma samples were collected in the tubes containing K2EDTA anticoagulant.

For protein profiling and repeatability experiments, sample preparation, including denaturation, alkylation, digestion, and peptide purification, was performed using the iST-BCT Kit 96x (PreOmics GmbH) both manually and with the PreON automation platform (software v.1.10.7, PreOmics GmbH). The rat plasma samples were run in quadruplicate on three different days (1st, 2nd, and 9th days) using the PreON platform. In addition, on day 9, rat plasma samples were prepared in quadruplicate manually. Detailed sample preparation of selected biofluids is described in Table 1. Additionally, Rat CSF and serum, human plasma, and mouse plasma were prepared in triplicates.

For equal sample loading, peptides from plasma and serum samples were further diluted 4.4-times with LC-LOAD. Biofluid peptides (2 µL of peptides) were analyzed in a 45-minute run using an EASY-nLC™ 1200 system (ThermoFisher Scientific) coupled with a timsTOF Pro (Bruker Daltonics) mass spectrometer (MS). The timsTOF was operated in data-dependent acquisition (DDA) mode with parallel accumulation—serial fragmentation (PASEF). MS and MS/MS data were acquired over the mass range from 100 to 1700 m/z. Acquired data were processed with MaxQuant (v.2.0.1.0) against the corresponding species-specific database: *Homo Sapiens* (up000005640, 17th March 2022), *Mus musculus* (Swiss-Prot DB, 21st February 2022), and *Rattus Norvegicus* (up000002494, 13th July 2022). The search criteria were set to 1% FDR at peptide and protein levels, with a minimum of one unique peptide per protein. Statistical analysis was performed using Perseus (v.2.0.3.0) and Microsoft Excel (v.16.65).

Table 1 | Biofluids sample preparation. Biofluids from human, mouse, and rat were processed manually and/or through the PreON platform. Samples were measured over three days.

Sample type	Sample volume (µL)	Dilutent (1xPBS) volume (µL)	Day 1	Day 2	Day 9	LC-LOAD volume (µL) to reconstitute peptides
Human plasma	2	8	PreON	---	---	100
Mouse plasma pool	2	8	PreON	---	---	100
Rat plasma pool	2	8	PreON	PreON	PreON / Manual	100
Rat serum pool	2	8	---	PreON	---	100
Rat CSF pool	10	---	---	PreON	---	10

Results and Discussion

Proteomic analysis of biofluids, such as plasma, serum, and CSF, is crucial for discovering early diagnostic biomarkers and monitoring disease progression on. The challenges for clinical proteomics are sample complexity, dynamic range, and the reproducibility of proteomics workflows to ensure reliable outcomes. Automating proteomics sample preparation reduces technical variability and increases reproducibility.[2]

The PreON, coupled with the iST-BCT sample preparation kit, can process a diverse range of biological fluid types in a fully automated fashion, as shown in Figure 2. In Figure 3, a principal component analysis (PCA) indicates a clear proteomics difference between rat blood fluids (plasma and serum) and rat CSF within a single species. The difference between plasma and serum proteomes is minimal, as levels of most components in serum and plasma proteomes show similar trends.[4]

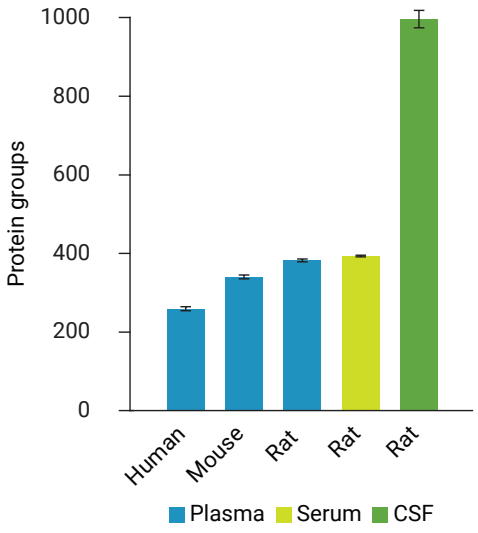

Figure 2 | Numbers of protein groups identified in the proteomic analysis of various biofluids collected from different species. Human plasma, rat plasma, and rat CSF were processed in quadruplicates. Mouse plasma and rat serum were processed in triplicate. The error bars indicate standard deviation.

Minimizing sample variability across multiple days or weeks is one of the challenges in clinical proteomics. Combining automation platforms, such as PreON, with a kit-based solution (iST-BCT) reduces errors caused by handling and pipetting during sample preparation, as shown in Figure 4. Samples processed using the PreON platform showed a 1.8-fold improvement in sample-to-sample variation at median %CV at 12.14, compared to manual processing with median manual %CV at 21.9, respectively (Figure 4).

To determine the intra- and inter-day variability of technical replicates, quadruplicate rat plasma samples were processed on days 1, 2, and 9. Our results indicate intra-day technical variability at median %CV 12.14 with day-to-day variation at day 1 = %CV 11.38, day 2 = 15.31, and day 9 = 12.14 (Figure 5). Inter-day variation median averaged %CV was 12.94. This result supports the advantage of processing biofluids using an automated sample preparation method.

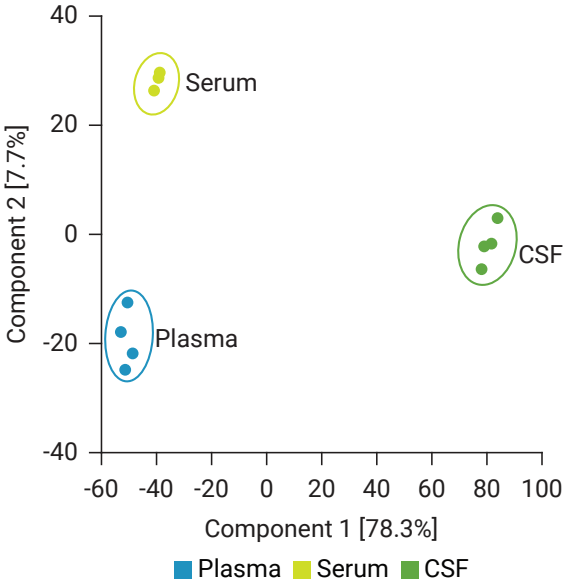

Figure 3 | Proteomic analysis of various biofluids.
Rat plasma and CSF were processed in quadruplicates, and Rat serum in triplicate. PCA plot indicates the clustering of samples base on type of biofluid. . Component 1 (x-axis) indicated a variance of 78.3%, with Component 2 (y-axis) variance of 7.7%.

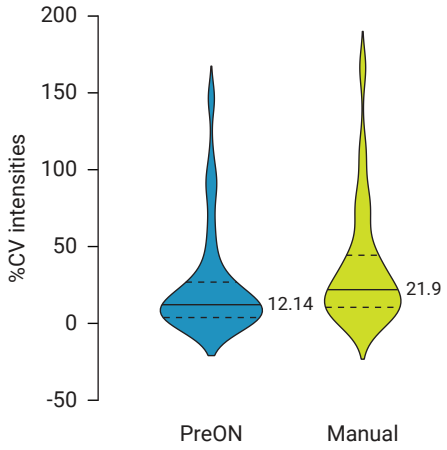

Figure 4 | Comparison of technical variability between manual and automated (PreON) sample preparation of rat plasma using the iST-BCT kit.
Plasma samples were processed in quadruplicate and in parallel using manual and automated iST-BCT workflows. The coefficient of variation represents measured peptide intensities.

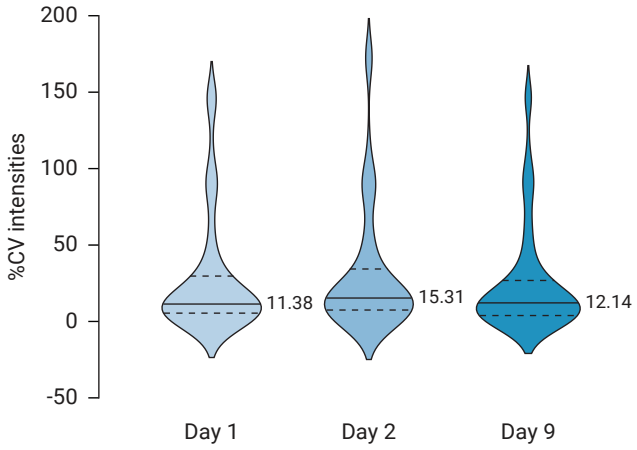

Figure 5 | Inter-day sample preparation variability of rat plasma processed with the iST-BCT kit on the PreON platform.
Plasma samples were processed in quadruplicate on three different days (Day 1, 2, and 9). The coefficient of variation represents measured peptide intensities. Averaged median %CV from day-to-day processing is 12.94%

Conclusions

Herein, we introduced a fully automated solution tailored to biofluids , such as plasma, serum, and CSF. It combines the iST-BCT sample preparation kit, utilizing the PreON automated platform to achieve clean, ready-to-measure peptides for LC-MS analysis. The presented results show highly reproducible sample preparation with shortened 5-minute hands-on time. This mid-throughput solution can be effortlessly integrated into laboratories seeking robust automation to further their discoveries of novel biomarkers or pathway regulation in oncology and other relevant clinical fields.

Products

Product	Manufacturer	Product Code
iST-BCT PreON 8x	PreOmics GmbH	P.O.00130
iST-BCT PreON 96x	PreOmics GmbH	P.O.00131
PreON	PreOmics GmbH	P.O.00069

Ordering information:

http://www.preomics.com/quote

order@preomics.com

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3. Application note: PreON automated sample preparation platform (https://uploads-ssl.webflow.com/5ea176125e0858f9bc861a53/5f50c9debc6f0e9b06a7f1d0_PreOmics_ApplicationNote_PreON_v6.pdf)

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