High-Throughput Proteomic Analysis of Stored Red Blood Cells from Non-Domestic Cat Species

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ABSTRACT

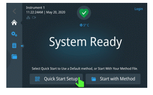
Purpose: Blood transfusions can be a life-saving treatment for animals that have become anemic due to trauma or disease. However, adverse, often life-threatening complications can occur if the donor and recipient blood types are not appropriately matched. In non-domesticated cat species, very little is known about the different blood types found in different species, making transfusions in most situations risky. Additionally, since non-domestic cats are often found in zoos where only a few individuals of each species are housed, there is a need to be able to either transfuse blood from one species to another, or store blood for prolonged periods of times in case of future emergencies.

Methods: Blood samples were obtained from more than 150 non-domestic cats, consisting of 18 different species, housed at AZA (Association of Zoos and Aquariums) accredited institutions across the United States. Samples from 36 of these animals were analyzed here. Fresh blood samples were aliquoted and stored in a clinical blood transfusion refrigerator for 0, 7, 14, or 28 days, after which the red blood cells were pelleted and stored at -80ºC until analysis. Samples were also cross matched to determine cross compatibility. Pelleted red blood cells were then prepared for bottom-up proteomic analysis using the newly introduced Thermo Scientific™ AccelerOme™ automated sample preparation platform that enables standardized, hands-off operation, and provides robust workflows for label free proteomics applications.

Results: The Thermo Scientific™ AccelerOme™ automated platform for sample preparation allowed for protein lysis, DNA removal, protein reduction, alkylation, protease digestion, and sample cleanup with no user intervention. Trypsin digested red blood cell samples were then analyzed using high-throughput, capillary flow LC-MS/MS analysis on a Thermo Scientific™ Orbitrap Exploris 480™ mass spectrometer, allowing for the analysis of more than 50 samples per day. Such methods allowed for the identification and label-free quantification of more than 2000 peptides per sample, mapping to approximately 500 protein groups, despite the high abundance of hemoglobin in the red blood cells. Results showed few missed cleavages and high reproducibility, suggesting efficient and complete digestion.

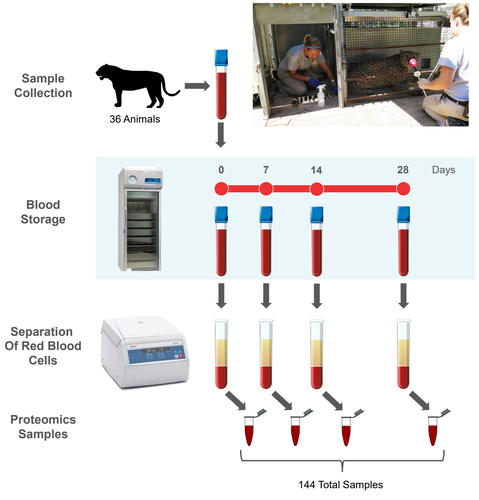
SAMPLE PREPARATION

The AccelerOme™ has an Experiment Designer software that guides the user through the experiment planning process to input sample names and assign study factors, values, and provides an estimate of statistical power. It also benefits from an integrated touchscreen display with user interface for instrument control and operation through a graphical wizard. The liquid handling robot has the capacity to process up to 36 label-free samples, 33 Thermo Scientific™ TMT11plex isobaric labeling reagent samples or 32 Thermo Scientific™ TMTpro™ 16plex label reagent samples per session. The AccelerOme has an automated and standardized workflows used to increase reproducibility and productivity which is part of an integrated workflow solution, from experiment design and sample preparation, to LC-MS analysis reducing training requirements while improving data quality.



STUDY DESIGN

Figure 2. Sample collection and processing summary. Blood was collected from animals housed at AZA institutions stored in a clinical blood transfusion refrigerator for 0, 1, 14, or 28 days after which the red blood cells will be pelleted and stored at -80ºC until proteomic analysis.



LC-MS ANALYSIS

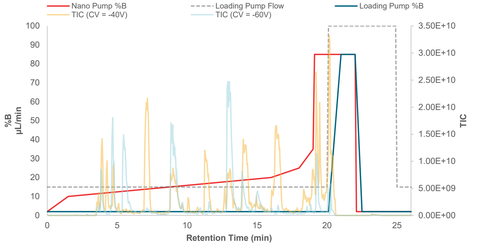
Figure 3. Hardware used for the LC-MS analysis of the red blood cells.

AccelerOme™ | UltiMate™ 3000 RLSCnano | Orbitrap Exploris™ 480 + FAIMS Pro™

LC Method

- Analytical Column: EASY-Spray™ PepMap™, 2 µm C18, 150 µm X 15 cm
- Mobile phases: [A] 0.1% FA in H_2O, [B] 0.1% FA in 100% ACN
- Nano Pump flow rate: 1.5 µL/min
- Trap and elute
- Column temperature: 50˚C
- 0.5 µL injections (0.5 µg on the column)

Figure 4. Overview of LC gradient used for high-throughput analysis. The 26-minute method allows for the analysis of ~55 samples per day and includes a high organic wash of the trap column to reduce carryover between injections.



MS Method

A data-dependent acquisition (DDA) acquisition scheme was used with a 60K Resolution MS^1 scan using a normalized AGC target of 300% (3e6) and 25 ms maximum injection time. A minimum intensity of 8.e3 was required to trigger an MS^2 scan, after which the precursor mass was dynamically excluded for a period of 60 seconds. Additional method parameters can be seen in Figure 5.

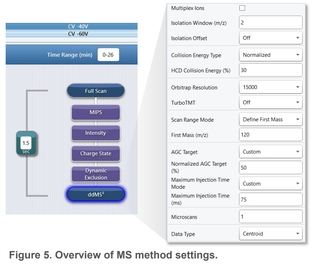


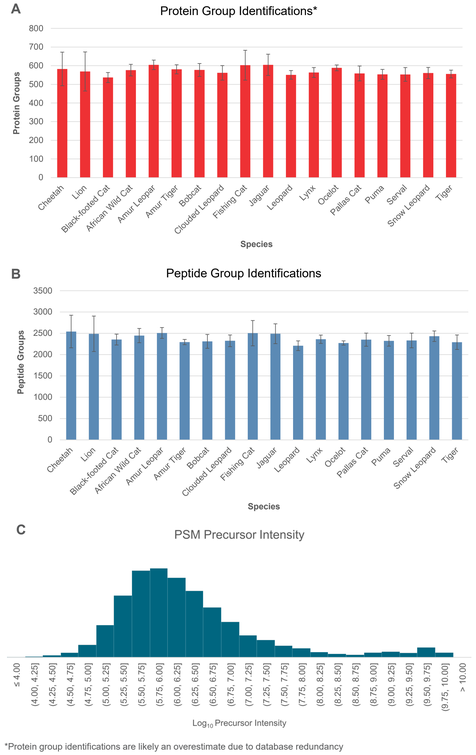
Figure 5. Overview of MS method settings.

DATA ANALYSIS

LC-MS data was searched using Proteome Discoverer™ version 2.5 against a protein database containing all entries in the UniProtKB database under the suborder Feliformia (taxonomic ID: 379583), including isoforms. The SEQUEST algorithm was used in tandem with INFERYS rescoring and Percolator for PSM validation.

RESULTS

Figure 6. Average protein group (A) and peptide group (B) identifications per sample across species. Protein group identification are likely an overestimation due to database redundancy. (C) Precursor intensity distribution for identified peptide spectral matches (PSMs).



*Protein group identifications are likely an overestimate due to database redundancy

Figure 7. Peptide spectral match (PSM) identifications across the two compensation voltages (CVs) used for the MS analysis

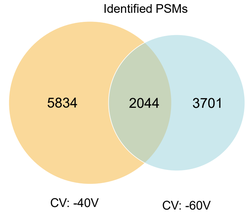
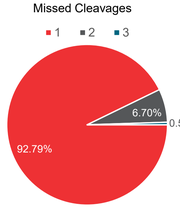


Figure 8. Number of missed cleavages for each identified peptide.



The AccelerOme automated sample preparation platform allowed for reproducible protein reduction, alkylation, digestion, and desalting with minimal user involvement. Despite the high dynamic range of the red blood cell proteome, which is dominated by several highly abundant proteins (such as hemoglobin), the identification of >2000 peptides (corresponding to approximately 500 proteins) was possible with a short, 26-minute method.

CONCLUSIONS

- AccelerOme™ offers simplified workflows with minimized user involvement and improved reproducibility through instrument functionality and automation. It helped increased efficiency and productivity through pre-built and validated sample preparation methods and reagents delivered in kit format, ensuring experiment democratization. AccelerOme™ reduced total cost of sample prep by eliminating the need for dedicated personnel for mass spectrometry sample preparation.
- Use of the UltiMate3000 RSLCnano system in conjunction with EASY-Spray PepMap , 2 µm C18, 150 µm X 15 cm allows for high-throughput analyses at low capillary flow rates (1.5 µL/min) resulting in the analysis of >50 samples per day.
- The Exploris 480 mass spectrometer with FAIMS Pro interface allows for the in-depth analysis of complex proteomic samples, even those with high dynamic range that are dominated by several highly abundant proteins.

ACKNOWLEDGEMENTS

[Waiting on collaborator for acknowledgements]

TRADEMARKS/LICENSING

Figure 1. Complete end to end proteomics workflow using the AccelerOme platform The experimental design and other metrics such peptide amount information are carried out along the process to be consumed for improved results and reduce operator errors.