Application note | AN004184

Software

Automated high-throughput proteomic analysis of stored blood cells from a large cohort of non-domestic felids

Authors

Kevin L. Schauer[1], Kevin Yang[1], Amirmansoor Hakimi[1], Eugen Damoc[2], Lily A.B. Parkinson[3]

[1]Thermo Fisher Scientific, San Jose, CA, USA

[2]Thermo Fisher Scientific, Bremen, Germany

[3]Brookfield Zoo Chicago, Brookfield, IL, USA

Keywords

Automated sample preparation, high-throughput proteomics, non-domestic felids, Orbitrap Astral mass spectrometer, AccelerOme platform, Proteome Discoverer software, Ardia Platform, biomarker

Goal

To establish a robust, automated workflow for high-throughput proteomic analysis of stored red blood cell samples from a large cohort of non-domestic felids, enabling in-depth biological insight while minimizing manual effort and variability. The workflow integrates the Thermo Scientific™ Ardia™ Platform for seamless data management and Thermo Scientific™ Proteome Discoverer™ software with CHIMERYS™ algorithm for deep, AI-enhanced analysis, enabling scalable and reproducible insights across large sample sets.

Application benefits

- High-throughput sample preparation with the Thermo Scientific™ AccelerOme™ automated sample preparation platform ensures standardized, hands-off processing with minimal variability across large sample sets
- Scalable Liquid Chromatography-Mass Spectrometry (LC-MS) acquisition workflow enabling the analysis of 100 samples per day with consistent performance, delivering deep proteome coverage across complex samples that span a broad dynamic range of protein abundances
- Automated data handling and analysis via the Ardia Platform and Proteome Discoverer software, streamlining data transfer, processing, and interpretation at scale

Introduction

Blood transfusions are critical interventions for animals suffering from anaemia due to trauma or disease. In non-domestic cat species, limited knowledge of blood types poses significant risks during transfusions. Zoos, often housing few individuals per species, face challenges in sourcing compatible blood, necessitating cross-species transfusions or prolonged blood storage. Domestic cat blood is not very stable during storage, so there is an interest in understanding if the same is true for other cat species. This study aims to analyze the proteomic profiles of stored blood cells from various non-domestic felid species to enhance understanding of blood compatibility and storage effects.

Experimental

Sample collection and storage

Blood samples were collected from 134 non-domestic cats, consisting of 18 different species, housed at the Association of Zoos and Aquariums (AZA) accredited institutions across the United States of America (Table 1).

Fresh blood samples were aliquoted and stored in a clinical blood transfusion refrigerator (4°C) for 0, 7, 14, or 28 days, after which the red blood cells were pelleted and stored at –80°C until analysis. Crossmatching was performed to assess compatibility.

Species	Common name	Individuals
Acinonyx jubatus	Cheetah	40
Felis caracal	Caracal	1
Felis lybica	African wildcat	1
Felis manul	Pallas cat	3
Felis nigripes	Black-footed cat	2
Leopardus pardalis	Ocelot	2
Leptailurus serval	Serval	3
Lynx canadensis	Canada lynx	9
Lynx rufus	Bobcat	3
Neofelis nebulosa	Clouded leopard	3
Panthera leo	Lion	22
Panthera onca	Jaguar	4
Panthera pardus	Leopard	2
Panthera tigris	Tiger	19
Prionailurus viverrinus	Fishing cat	3
Puma concolor	Cougar	7
Uncia uncia	Snow leopard	10

Table 1: Species makeup of animals from which blood was collected for this study.

Sample preparation

Pelleted red blood cells were prepared for bottom-up proteomic analysis using the AccelerOme automated sample preparation platform (**P/N C0960-01-00109**), enabling standardized, hands-off processing. Allowing for protein lysis, DNA removal, protein reduction, alkylation, protease digestion and sample cleanup with no user intervention.

The AccelerOme Experiment Designer software guides the user through the experiment planning process, allowing input of sample names and assignment of study factors, and provides an estimate of statistical power. It can be accessed at a remote computer, and the prepared method can be imported directly to the AccelerOme platform. After the experiment is defined, a graphical wizard with an integrated touchscreen display on the AccelerOme platform directs set up and operation, simplifying sample preparation.

The AccelerOme platform has the capacity to process up to 36 label-free samples, 33 Thermo Scientific™ TMT11plex™ isobaric labelling reagent samples, or two sets of Thermo Scientific™ TMTpro™ 16plex™ label reagent samples per session. It automates and standardizes workflows to enhance reproducibility and productivity, serving as part of an integrated solution from experiment design and sample preparation to LC-MS analysis, thereby reducing training requirements and improving data quality (Figure 1).

In this study, the blood cell pellets were thawed on ice and subsequently lysed by resuspending in 500 µL of AccelerOme lysis buffer followed by vortexing for 5 minutes. A 75 µg aliquot from each sample was then diluted to a volume of 50 µL with lysis buffer loaded into the AccelerOme 96-well plate for digestion, clean up, and measurement of the resulting peptide amounts with the AccelerOme Label-Free MS Sample Prep Kit (**P/N A50945**).

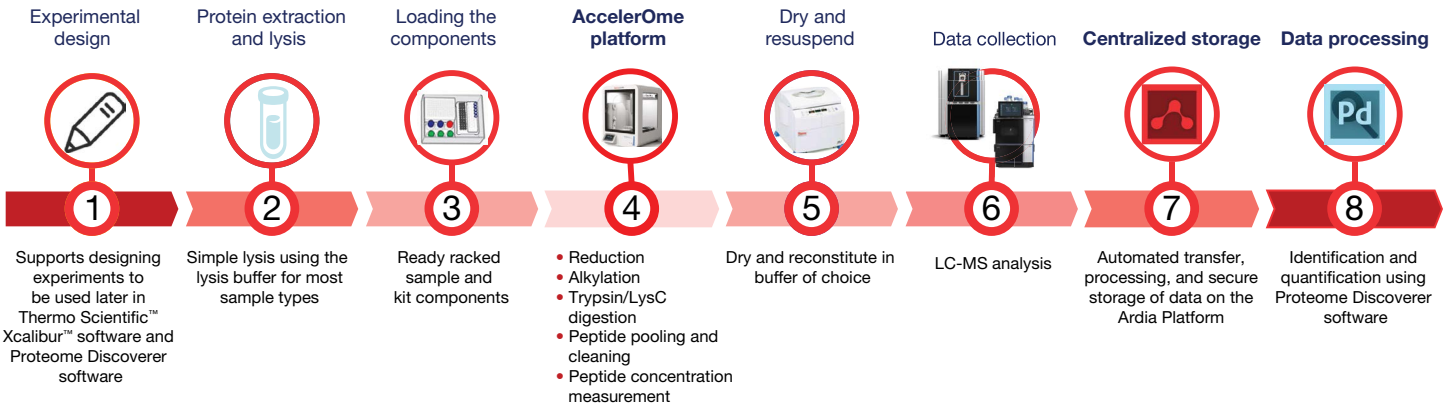


Figure 1. Complete end-to-end proteomics workflow. Automated sample preparation with the AccelerOme liquid handling robot and automated transfer, processing, and secure storage of data on the Ardia Platform.

LC-MS analysis

The trypsin digested red blood cells were then analyzed using high-throughput, capillary flow LC-MS/MS analysis.

Chromatographic separation was performed with the Thermo Scientific™ Vanquish™ Neo UHPLC system (**P/N VN-S10-A-01**) equipped with a Thermo Scientific™ EASY-Spray™ PepMap™ C18 column (2 µm, 150 µm × 15 cm) (**P/N ES906**) designed to provide robust and high-resolution peptide separations. Peptide samples (0.2 µL each, corresponding to 200 ng on-column) were injected at a flow rate of 2.0–2.5 µL/min. The mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in 80% acetonitrile (B). The column temperature was maintained at 50°C to ensure optimal chromatographic performance. A trap-and-elute injection scheme was employed, utilizing the ZebraWash procedure to minimize trap column carryover and enhance analytical robustness. The total method duration was 13 minutes, supplemented by approximately 3 minutes for loading and equilibration, allowing for the analysis of 100 samples per day. Each sample underwent duplicate injections to ensure reproducibility and data reliability.

Mass spectrometric analysis was performed on a Thermo Scientific™ Orbitrap™ Astral MS (**P/N BRE725600**). The method consisted of a data-independent acquisition (DIA) scheme consisting of 199, 3 m/z windows that spanned from 380-980 m/z. MS1 scans were collected every 0.6 seconds at 240K resolution with a normalized AGC target of 500%, and 3 ms maximum injection time. Additional method parameters can be seen in Table 2.

Data independent acquisition properties	
Precursor mass range (m/z)	380–980
Isolation window (m/z)	3
Window overlap	0
HCD collision energies (%)	Normalized
Detector type	Astral
TMT	Off
Scan range (m/z)	150–2000
RF lens %	40
Normalized AGC target (%)	500
Polarity	Positive
Loop control	Time
Time (sec.)	0.6

Table 2: Orbitrap Astral MS acquisition parameters.

Data processing

Acquired data was written directly to the **Ardia Platform** via connection of Thermo Scientific™ Xcalibur™ 4.7 software and processed using Proteome Discoverer 3.1 software (**P/N CSW0064764**).

The Proteome Discoverer software processing method was specified in the Xcalibur sequence, allowing data processing as described below to automatically begin immediately post-acquisition (Figure 2).

Connection of the LC-MS system to the Ardia Platform also allowed for remote, real-time monitoring of acquisition throughout data collection. The Ardia instruments application provided real-time estimates for the remaining acquisition time and showed real-time chromatograms as data collection proceeded.

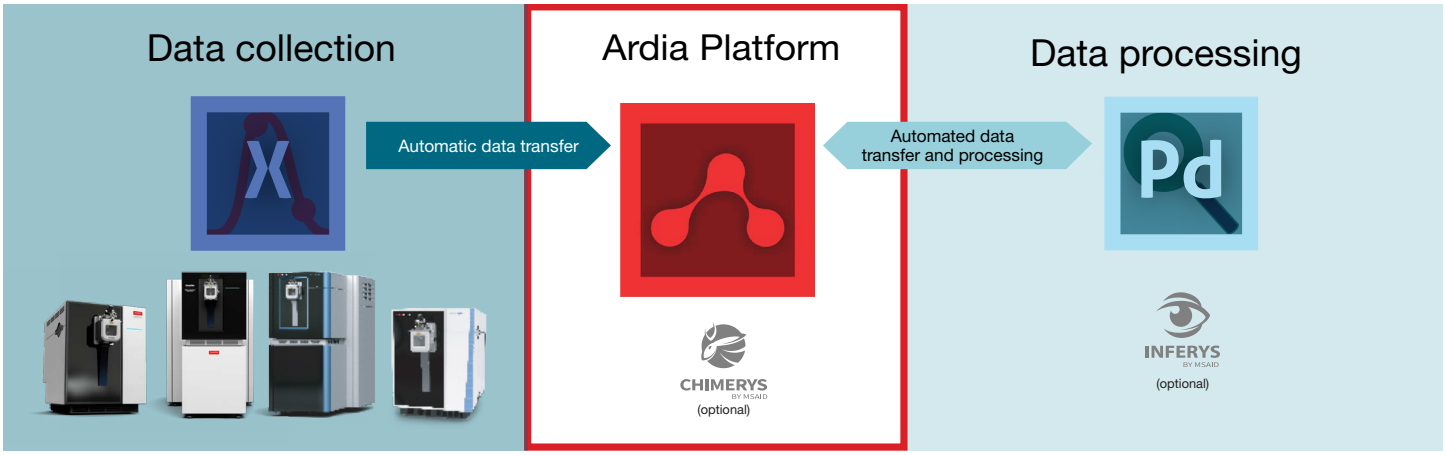


Figure 2. Data is acquired and automatically uploaded to the Ardia platform. The data is subsequently processed in Proteome Discoverer software where it is automatically triggered as each injection completes, and results are automatically sent back to the platform for secure storage.

Data was searched using the CHIMERYS 2.0 search algorithm by MSAID, which is integrated into Proteome Discoverer 3.1 software. The CHIMERYS algorithm runs directly on the Ardia Advanced server, so no cloud subscription is required.

The Ardia server allows for up to 20 simultaneous CHIMERYS searches in parallel without a decrease in performance (Figure 3). A database downloaded from UniProt that contained all protein entries (including sub-taxa) from the Feliformia suborder was used for all searches. A small pilot study consisting of two samples from each species was analyzed prior to the start of the main searches to produce a CHIMERYS inclusion file, allowing for Match Between Runs (MBR) to be performed while analyzing data on a file-by-file basis. Upon completion of data acquisition and processing, a single multiconsensus was run to combine all analyses into a single result file.

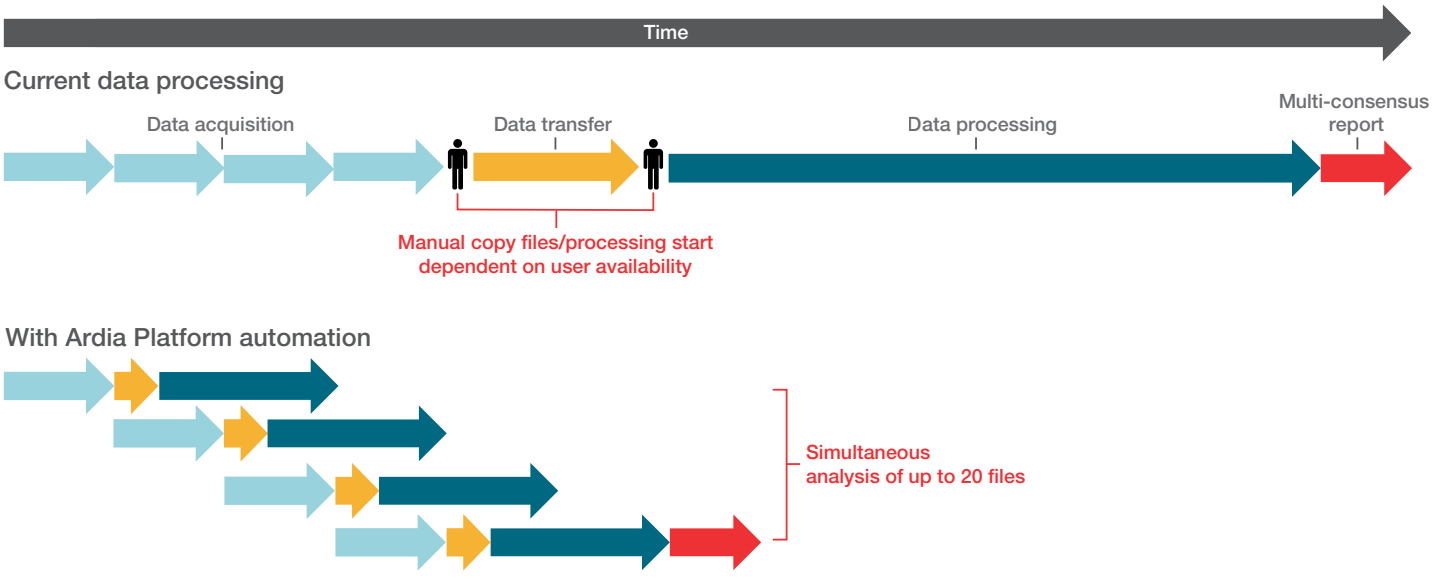
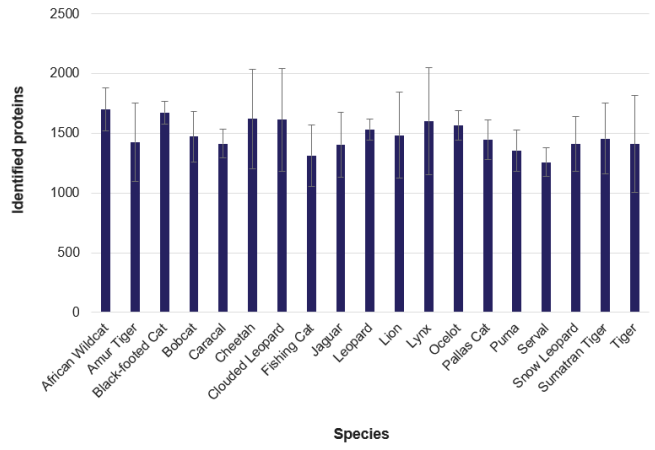


Figure 3. Schematic illustrating how multi-stream multitasking of processes enhanced with automation functions reduces wait time and increases data analysis.

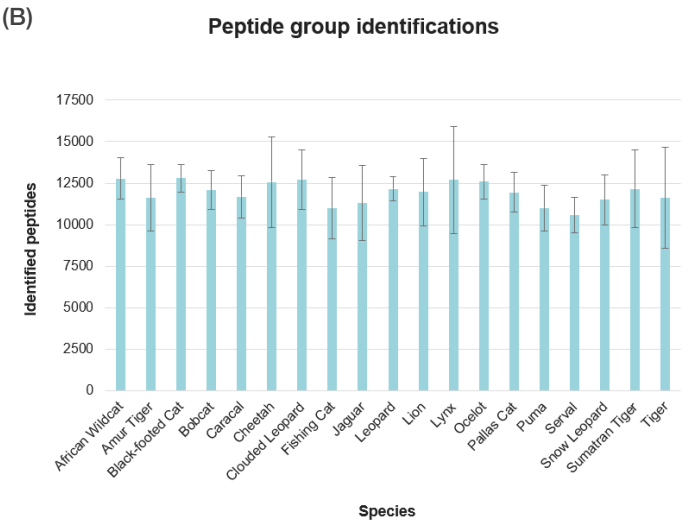
Results

In total, 1,072 injections were completed in this study using samples derived from 134 individual cats. On average, more than 10,000 peptides were identified in each sample, mapping to approximately 1,500 proteins (Figures 4a and 4b). However, the actual number of unique proteins is likely lower as there was significant redundancy in the database used as it contained entries from numerous species. No significant changes in sensitivity were observed across the 16 days of acquisition (Figure 4c), and protein signal intensities spanning more than 7 orders of magnitude were observed (Figure 4d). Principle component analysis (PCA) showed clear clustering of samples derived from each species, which closely mimicked the phylogenetic relationships of the species studied (Figure 5).

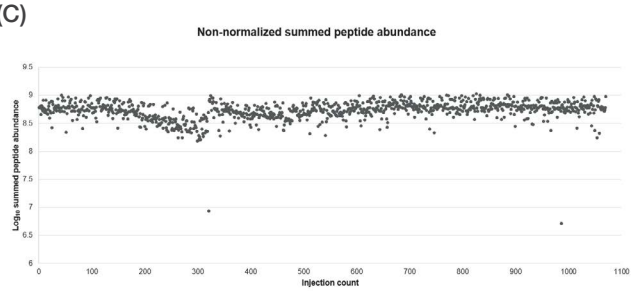
(A)



(B)



(C)



(D)

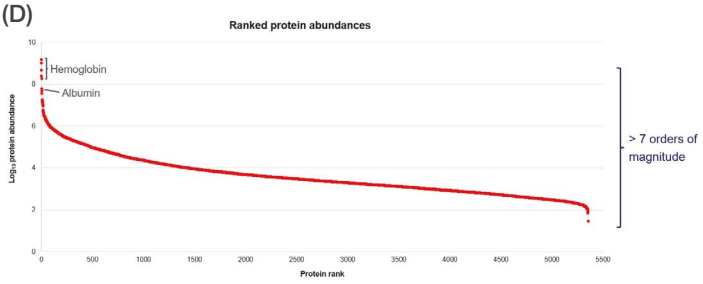


Figure 4. Average protein group (A) and peptide group (B) identifications per sample across species. (C) Non-normalized sum protein abundances across all samples analyzed. (D) Ranked protein abundances of quantified proteins from the 156 cheetah samples analyzed. Protein signal intensities spanned more than 7 orders of magnitude.

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

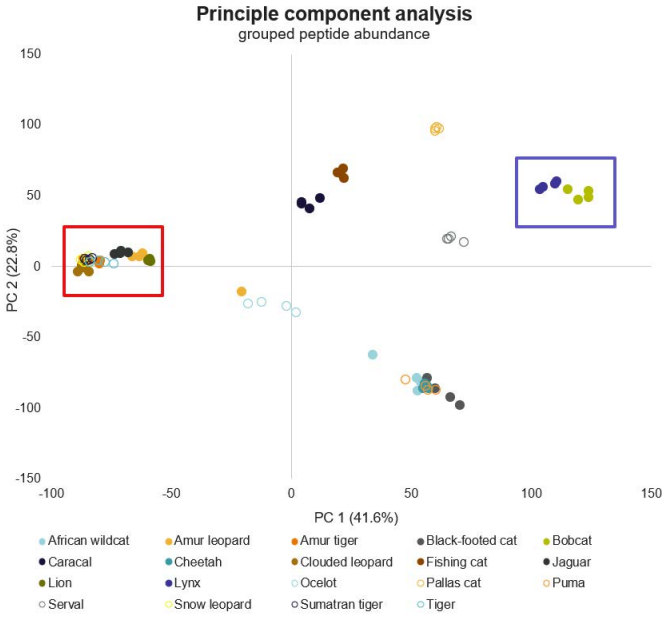


Figure 5. Principle component analysis (PCA) plot derived from grouped peptide abundances of all analyzed samples. Clear clustering within species and amongst closely related species is observed.

Conclusions

- Proteome Discoverer software powered by the Ardia Platform allows for the analysis and management of large cohort, high-throughput analyses. Even for bioinformatically challenging, multi-species studies.
- Automation of acquisition via Xcalibur software and processing via Proteome Discoverer software and CHIMERYS algorithm with the Ardia Platform simplifies data management and reduces processing time for large cohort studies.
- The Vanquish Neo UHPLC and Orbitrap Astral MS combination is an ideal platform for high-throughput proteomics studies requiring analysis of 100+ samples per day with high depth of coverage.
- The Orbitrap Astral MS is well suited for experiments where high dynamic range is required. Proteins with signal intensities spanning more than 7 orders of magnitude were observed here.
- The AccelerOme platform offers simplified sample preparation 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.

Acknowledgements

The authors express gratitude to the AZA institutions contributing blood samples and acknowledge the University of Georgia Small Animal Medicine and Surgery (SAMS) grant for funding. Special thanks to Kathy Hoover for her invaluable assistance in sample analysis and logistics.

Learn more at **thermofisher.com/ardiaproteomics**

AN004184-EN 1125