New Approach for Room Temperature Stabilization and Storage of High Quality Plasma Samples

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Introduction

Researchers need access to high quality biological samples to be used both for clinical and research purposes. Because of that, a wide range of primary samples and their subproducts may be processed and stored for either short or long-term. The most extended method for preservation of plasma or serum samples is ultra-low temperature (ULT) freezing at -80 ºC. However, maintaining those protective conditions implies high maintenance costs, large spaces, constant energy supply and safety measures to minimize the risk of missing collections.

Here, *300K Solutions* proposes an alternative approach for room temperature (RT) preservation based on plasma/serum drying and stabilization that has the potential to be a real alternative to the current ULT storage, preserving these samples with good quality.

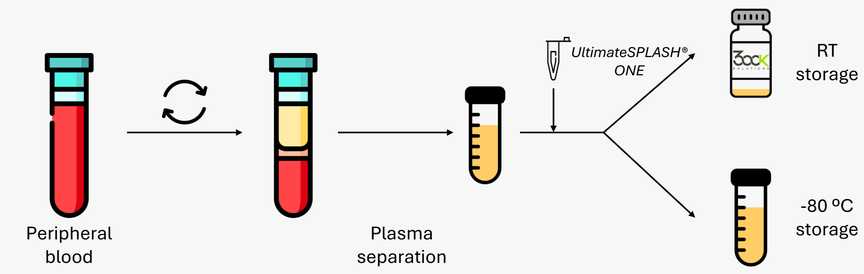
Objectives

- Evaluate the applicability of *300K Solutions* stabilization technology for RT storage of plasma samples.
- Compare the lipidomics, metabolomics and proteomics of plasma stabilized, dried and stored at RT with those of plasma stored under "gold standard" conditions (frozen at -80 ºC).

Materials and Methods

Plasma samples were obtained from peripheral blood of 4 different donors following strictly the preanalytical procedures for the handling of these type of samples. Then, this plasma was aliquoted and *UltimateSPLASH® ONE Internal Standard for Lipidomic Analysis (Avanti Polar lipids)* were added to each aliquot following manufacturer's instructions.

Afterwards, one half of the generated aliquots was frozen and stored at -80 ºC, and the other half was dried and stabilized at RT using the *Sample Stabilization System (S^3)* of *300K Solutions SL*.



After 6 months of storage, one aliquot of each donor and condition was thawed (-80 ºC storage) or rehydrated (RT storage). These samples were then subjected to three different analysis with liquid chromatography/mass spectrometry (LC/MS) by *oloBion SL (Barcelona, Spain):*

- Lipidomics: each plasma sample (25 µL) was mixed and shaken with 765 µL of cold methanol/MTBE mixture. Then, 165 µL of 10% methanol with internal standards was added, shaken, and centrifuged. For the lipidomic profiling, 100 µL of the upper organic phase was collected, evaporated and resuspended using 100 µL methanol. After shaking and centrifugation, the sample was used for LC-MS analysis. The lipids were separated on an *ACQUITY UPLC BEH C18 column* maintained at 65°C and coupled to *a ZenoTOF 7600 system (SCIEX).* The sample was injected at 4 µL in ESI negative mode.
- Metabolomics: each plasma sample (25 µL) was mixed and shaken with 765 µL of cold methanol/MTBE mixture containing a mixture of internal standards. Then, 165 µL of 10% methanol with internal standards was added, shaken, and centrifuged. For the metabolomic profiling, 70 µL of the bottom aqueous phase was collected, evaporated, and resuspended using 70 µL acetonitrile/water. After shaking and centrifugation, the sample was analyzed using the HILIC metabolomics platform. Another 70 µL aliquot of the bottom aqueous phase was mixed with isopropanol/acetonitrile, shaken, centrifuged, and evaporated. The dry extracts were resuspended in methanol with internal standards. After shaking and centrifugation, the sample was analyzed using the RPLC metabolomics platform. The polar metabolites were separated on a *Waters ACQUITY UPLC C18 BEH Amide and ACQUITY UPLC HSS T3 columns* maintained at 45°C and coupled to an *Agilent 1290 Infinity UHPLC (Agilent Technologies).* The samples were injected at 4 µL for ESI positive and negative modes, respectively.
- Proteomics: plasma samples (2 µl) were extracted by using the *iST sample preparation kit of PREOMICS*. The extracted peptides were separated on *Kinetex XB-C18 column* maintained at 40°C by using *micro flow M5 system (SCIEX)* and coupled to *a ZenoTOF 7600 system (SCIEX).*

All omics data were processed by *oloMAP 2.0* (*oloBion SL, Barcelona, Spain*)

Conclusions

- Freeze-drying stabilization of samples with *300K Solutions* technology is a suitable method for preserving plasma at RT.
- The results obtained in the lipidomics, metabolomics and proteomics analysis performed, after 6 months, in dried plasma samples were similar to those obtained from frozen plasma samples.
- The technology developed by *300K Solutions* allows the use of plasma samples store at RT for complex downstream applications.

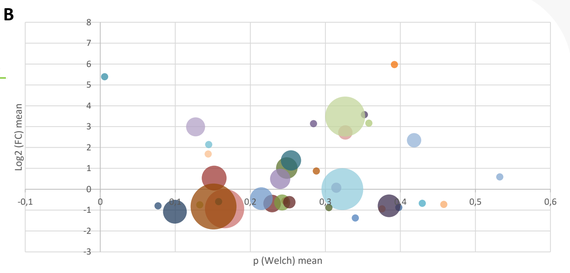
Results

The lipidomics analysis in freeze-dried and frozen plasma samples from all 4 donors identified a total of 548 lipids distributed in 6 categories, 23 main classes and 51 subclasses (A). When compared, all lipid subclasses showed no significant differences between freeze-dried and frozen samples (pWelch <0,05) (B). Moreover, most of the lipids identified and quantified showed no significant differences between freeze-dried and frozen samples, with the exception of 11 lipids (pWelch <0,05) (C).

A

Total Count	Category count	Main Class Count	Subclass Count
548	6	23	51

B



C

Lipid Subclass	Lipids with p<0,05	p (Welch) mean
Ether-linked lysophosphatidylcholine	1	0,003
Monoacylglycerol	1	0,006
Oxidized phosphatidylcholine	2	0,018
Oidized triglyceride	1	0,048
Phospatidylethanolamine	1	0,048
Triacylglycerol	5	0,032

The metabolomics analysis in freeze-dried and frozen plasma samples from all 4 donors identified a total of 203 metabolites distributed in 10 superclasses and 34 classes (D). This analysis was made without considering some metabolites of organic oxygen compounds and lipids and lipid-like molecules superclasses. When compared, all metabolic superclasses showed no significant differences between freeze-dried and frozen samples (pWelch <0,05) (E). Moreover, most of the metabolites identified and quantified showed no significant differences between freeze-dried and frozen samples, with the exception of 27 metabolites (pWelch <0,05) (F).

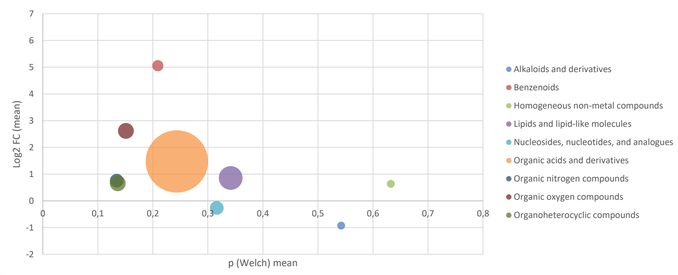
D

Total Count	Superclass Count	Class Count
80	10	34

F

Superclass	Metabolites with p<0,05	p (Welch) mean
Benzenoids	1	0,000
Lipids and lipid-like molecules	1	0,021
Nucleosides, nucleotides and analogues	1	0,002
Organic oxygen compounds	2	0,036
Organic nitrogen compounds	2	0,007
Organoheterocyclic compounds	1	0,037
Organic acids and derivatives	19	0,011

E

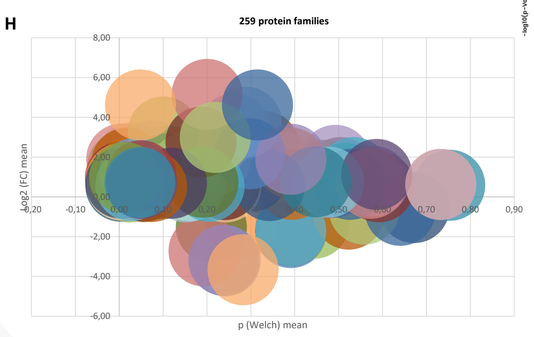


Finally, the proteomics analysis in freeze-dried and frozen plasma samples from all 4 donors identified a total of 285 proteins distributed in 259 families (G). When compared, 32 proteins showed significant differences between freeze-dried and frozen samples (pWelch <0,05) (H). These 32 proteins showed lower levels in frozen samples (I).

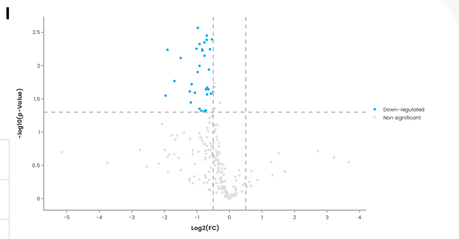
G

Total Count	Family count
285	259

H



I



1. Apolipoprotein L1
2. Inter-alpha-trypsin inhibitor heavy chain H2
3. Clusterin
4. Immunoglobulin lambda constant 3
5. N-acetylmuramoyl-L-alanine amidase
6. Fetuin-B
7. Trypsin
8. Thyroxine-binding globulin
9. Immunoglobulin lambda constant 7
10. Apolipoprotein D
11. Apolipoprotein B-100
12. Coagulation factor V
13. Plasma kallikrein
14. Serotransferrin
15. Transthyretin
16. Complement C1q subcomponent subunit C
17. Complement C1q subcomponent subunit A
18. Apolipoprotein C-II
19. Apolipoprotein A-II
20. Immunoglobulin heavy constant gamma 2
21. Immunoglobulin heavy constant gamma 1
22. Immunoglobulin kappa constant
23. Immunoglobulin heavy variable 3-33
24. Beta-2-glycoprotein 1
25. Zinc-alpha-2-glycoprotein
26. Immunoglobulin lambda-like polypeptide 5
27. Immunoglobulin kappa variable 1-5
28. Complement factor H-related protein 1
29. Extracellular matrix protein 1
30. Plasminogen
31. Alpha-2-antiplasmin
32. Serum paraoxonase/arylesterase 1

Project funded by the European Regional Development Fund (ERDF) of the European Union and the Regional Government of Castilla y León, through the Instituto para la Competitividad Empresarial de Castilla y León (ICE), with the aim of promoting research, technological development, and innovation. (Grant nº: 04/18/SA/0055).

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