Novel Dry Technology for Stabilization and Room Temperature Storage of Tissue Samples for Genomic Studies

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Introduction

It is well known that nucleic acid extraction from formalin-fixed, paraffin-embedded (FFPE) tissues is a challenging procedure due to the need to remove the paraffin and counteract covalent protein-DNA interactions that result from the fixation process. Because of that, FFPE samples showed an increase in degradation and a loss of high molecular weight DNA in comparison with matched fresh and frozen specimens, being the later the recommended storage for this downstream application. However, this Ultra Low Temperature (ULT) storage presents several disadvantages such us the risk of sample loss, high cost and heavy maintenance.

Here, 300K Solutions proposes an alternative approach for room temperature (RT) preservation based on tissue drying stabilization that has the potential to become a real alternative to the current ULT storage to obtain good quality DNA and RNA from tissue samples.

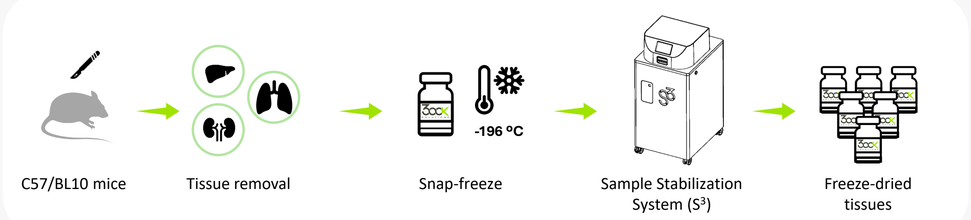
Objectives

- To evaluate the applicability of 300K Solutions stabilization technology for RT storage of tissue samples.
- To obtain good-quality DNA and RNA from dried tissue samples.
- To assess the suitability of dried tissue samples for histology and immunohistochemistry (IHC) analysis.

Materials and Methods

Samples of 3 different tissues (lung, kidney and liver) were obtained from five C57/BL10 mice following the Code of Good Scientific Practices of the IBMCC-CIC (USAL-CSIC). Then, these tissue samples with a ¼ kidney equivalent size, were embedded in 4 different stabilization buffer conditions to facilite and protect the tissue during the freeze-drying process.

Afterwards, the embedded tissues were snap-frozen by immersion in liquid nitrogen for 50 seconds. Finally, samples were freeze-dried for 48 h for their stabilization using the *Sample Stabilization System (S^3)* developed by *300K Solutions SL*.



After lyophilization and 1, 3, 4 and 5 weeks of room temperature (RT) storage, a dried sample of each condition and tissue (from the same mouse) was used for nucleic acid extraction (DNA and RNA) by organic solvent method, and then subjected to a basic quality control (QC) procedure following the Proficiency Standards stablished by the *International Society for Biological and Environmental Repositories (ISBER*).

- DNA/RNA purity by spectrophotometry.
- Determination of DNA/RNA integrity by agarose gel electrophoresis.
- DNA/RNA integrity analysis by Agilent 2200 TapeStation System

Finally, in collaboration with the *Compared Molecular Pathology Service (PMC)* at the *Cancer Research Center (CIC) of University of Salamanca (Spain),* another dried sample of each condition and tissue was rehydrated and used for histology (H&E stain) and IHC (Ki67, CD31 and Vimentin) analysis following the standard fixation protocol used with FFPE tissues.

Conclusions

- Freeze-drying stabilization of samples with *300K Solutions* technology is a suitable method for tissue preservation at RT.
- The technology here proposed allows the extraction of good-quality DNA from dried tissue samples stored at RT for its potential use in advanced genomic applications.
- RNA extracted from certain tissues after RT stabilization using *300K Solutions* technology presents enough quality for downstream applications.
- Freeze-dried tissue samples are unsuitable for histology but suitable for IHC.
- Further studies are required for determining which lyoprotectant is the ideal for the preservation of both good-quality DNA and RNA independently of the tissue selected.

Results

To evaluate if our stabilization technology allowed long-term stability during RT storage of freeze-dried tissue samples, we compared DNA and RNA quality right after the freeze-drying process and after 5 weeks of RT storage. This QC assessment is focussed on 2 different parameters: purity and integrity.

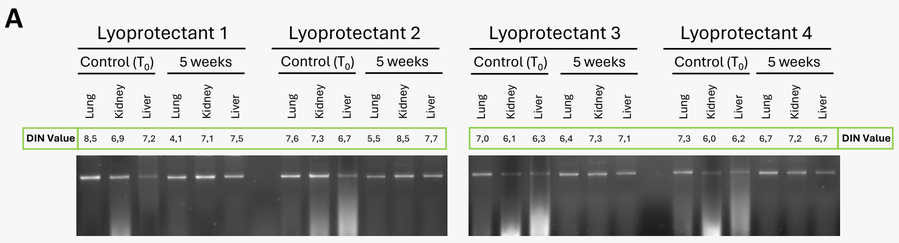
First, we assessed DNA/RNA purity by spectrophotometry and observed that tissues stored under all four different conditions, showed A260/280 ratio values above 1.6 even after 5 weeks of RT storage, which indicates absence of massive protein contamination.

DNA Sample ID	Condition	A260/280 Control (T_0)	A260/280 5 weeks
Lung	Lyoprotectant 1	2,03	2,07
Kidney		2,11	2,06
Liver		2,08	2,07
Lung	Lyoprotectant 2	2,05	2,07
Kidney		2,10	2,08
Liver		2,11	2,09
Lung	Lyoprotectant 3	2,08	2,11
Kidney		2,11	2,10
Liver		2,12	2,10
Lung	Lyoprotectant 4	2,10	2,06
Kidney		2,11	2,09
Liver		2,12	2,10

RNA Sample ID	Condition	A260/280 Control (T_0)	A260/280 5 weeks
Lung	Lyoprotectant 1	1,86	1,95
Kidney		2,03	2,05
Liver		1,94	1,97
Lung	Lyoprotectant 2	1,85	1,93
Kidney		2,01	2,05
Liver		1,96	1,98
Lung	Lyoprotectant 3	1,80	1,92
Kidney		2,02	2,07
Liver		1,95	1,97
Lung	Lyoprotectant 4	1,89	1,94
Kidney		2,04	2,06
Liver		1,95	2,01

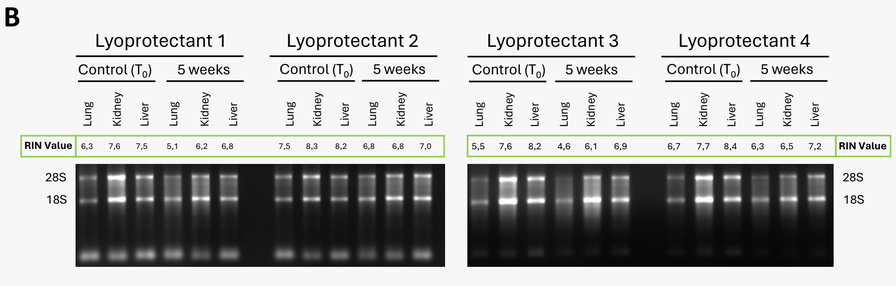
Second, using agarose gel electrophoresis we assessed DNA and RNA integrity in all samples. On one hand, regarding DNA integrity, we observed that despite the presence of a smear mostly in the control samples, a define band corresponding to high-weight DNA was detectable in all samples and conditions, even after 5 weeks of RT storage (A). Moreover, most of the DNA integrity values (DIN) were ≥ 6, confirming their good quality. Interestingly, only lung samples stored under two of the four stabilization conditions (lyoprotectants 1 and 2) showed partial degradation as showed by their DIN values under 6 (A).

A



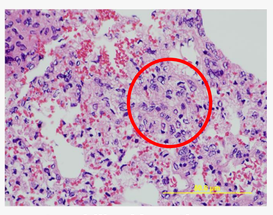
On the other hand, regarding RNA integrity, we observed define bands corresponding to 28S and 18S rRNA in all samples and conditions, even after 5 weeks of RT storage, with the exception of lung samples using lyoprotectants 3 and 4 that showed degradation of the 28S band (B). Moreover, most of the RNA integrity values (RIN) were > 6, confirming their good quality. Once again, lung samples stored under two of the four stabilization conditions (lyoprotectants 1 and 3) showed partial degradation as showed by their RIN values under 6 (B).

B



Additionally, we assessed the suitability of our technology for histology and IHC analysis. First, we performed the commonly used hematoxylin & eosin (H&E) staining and observed that, although the tissue structure is largely preserved after lyophilization, it is also possible that this process leads to some areas with nuclear inclusions (red circle) similar to the alterations we can observe in non lyophilized samples when we have denser areas such as tumors (C).

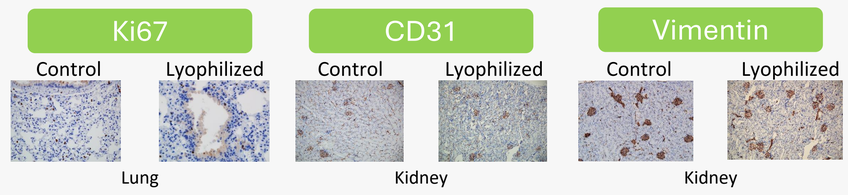
C



Lyophilized lung tissue

Second, we performed IHC analysis using three widely used biomarkers such as Ki67, CD31 and Vimentin, and demonstrate that lyophilized tissue samples are suitable for this kind of this technique (D).

D



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