Novel Dry Technology for Stabilization and Room Temperature Storage of Whole Peripheral Blood Samples

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Introduction

Peripheral Blood (PB) is a widely used biospecimen that plays a key role in clinical research. Its various components provide a multitude of possible applications such as immune profiling, proteomics or genomic studies, among others. With such versatility in its uses, it is desirable that blood could be optimally collected and stored since some of the previously mentioned applications require fresh blood samples for accurate results, and that is not always possible due to time or transport constraints. In this context, although there are several commercially available solutions to preserve cells fixed for some days, the most common choice is the cryopreservation of peripheral blood mononuclear cells (PBMCs). However, this approach requires specialized ultra-low freezer facilities, complex shipment procedures, and no exempt from the possibility of losing the samples. Moreover, there are studies describing a selective cell loss during PBMCs isolation, that together with the variability in the recovery after thawing the samples, may induce a bias in subpopulation distribution.

To address these limitations, here we propose an innovative approach based on a precision freeze-drying technique to stabilize samples that allows optimal storage conditions at room temperature (RT) of whole PB in a standard process that aims to minimize pre-analytical variations.

Objetive

- To evaluate the applicability of 300K Solutions stabilization technology for RT storage of PB samples.
- To assess the suitability of dried PB samples for flow cytometry analysis.
- To obtain good-quality DNA from dried PB samples.

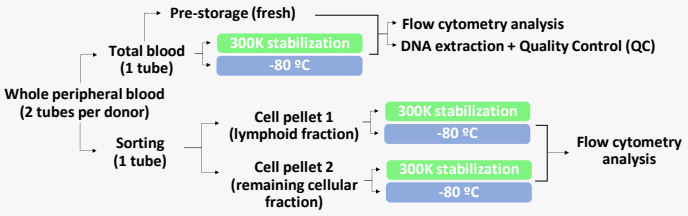
Materials and Methods

6 PB samples of healthy donors were obtained from *Centro de Hemoterapia y Hemodonación de Castilla y León (CHHCyL)*. From each one of these 6 donors, fresh sample analysis consisted of cell count by flow cytometry using TruCount beads (*BD Biosciences*) in a FACSCanto (*BD Biosciences*), followed by the analysis of the frequency of the different cell subpopulations with the following markers: CD4-PB, CD45-PO, CD8-FITC, Lambda-FITC, CD56-PE, Kappa-PE, CD19-PECy7 and CD3 APC, all purchased from *Exbio Pharma*. From each total PB sample, part was frozen and stored at -80ºC in 1 mL aliquots, and part was freeze-dried in 0,5mL aliquots using *300K solutions* stabilization technology. In addition to total PB analysis and storage, samples from each donor were also subjected to sorting and lymphoid fractions and rest of cells fractions were obtained (10^6 cells per aliquot). Same as with total PB, part was freeze-dried, and part was stored at -80ºC.

After 2 weeks, dried aliquots were re-hydrate and frozen aliquots were thawed for a parallel analysis of the same variables studied in the fresh sample: absolute counts and frequency of lymphoid and myeloid subpopulations.

For all three conditions (fresh, frozen and dried aliquots) flow cytometry analysis was performed using Infinicyt® software (*BD Biosciences*).

Furthermore, using the phenol/chloroform extraction method, DNA from PB samples (fresh, frozen and dried) of all 6 donors was obtained and its quality assessed following the Proficiency Standards stablished by the *International Society for Biological and Environmental Repositories (ISBER)*.



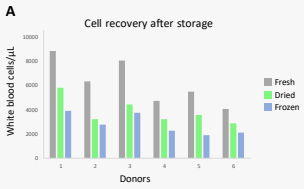
Conclusions

- Freeze-drying stabilization of samples with *300K solutions* technology is a suitable method for preserving Peripheral Blood at RT.
- Frequency of subpopulations is not altered, and membrane markers can be easily identified by flow cytometry.
- The technology here proposed allows the extraction of good-quality DNA from dried PB samples stored at RT.
- Further stability studies are required for determining the time these samples can be stored without affecting the sample quality.

Results

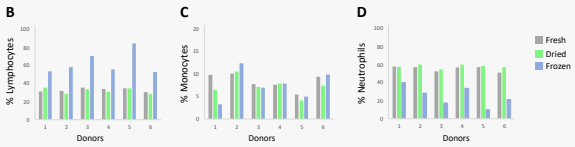
Cell recovery

The absolute count analysis performed in fresh, dried and frozen samples showed, when compared with the fresh sample, a decrease of cell counts in dried samples (62,53%) which was more pronounced in frozen samples (44,56%) (A).

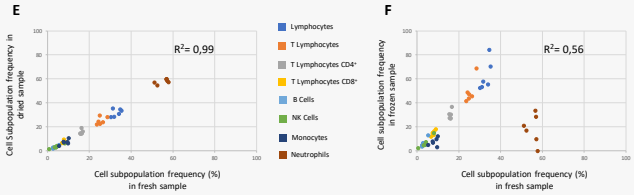


Relative distribution of cell subpopulations

When main lymphoid and myeloid samples were separately analyzed to compare the frequency of each subpopulation within the total sample, a selective loss of myeloid cells was observed with decreased values of neutrophils in the frozen samples, resulting in an imbalance on the rest of subpopulations (D). On the other hand, dried cells kept similar frequencies to fresh samples (B-D).



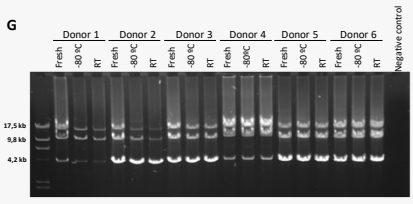
Importantly, statistical analysis showed an excellent correlation (R^2>0,9) in dried cell subpopulations vs fresh subpopulations (E) while a lower one in frozen samples vs fresh samples (F).



Interestingly, when the same analysis was performed in cell pellets of sorted fractions, similar patterns in cell loss and frequencies were observed both in fresh, dried and frozen (data not shown) samples.

DNA preservation in dried peripheral blood

Finally, we assessed if this stabilization solution allows the extraction of good-quality DNA from dried PB samples by comparing its purity, integrity and functionality with that of DNA extracted from fresh and frozen samples. QC of samples showed good purity and integrity when assessed by spectrophotometry and agarose gel electrophoresis, respectively (data not shown). As shown in the figure below, we additionally perform a multiplex long PCR and all samples displayed a 17.5 kb band (G), confirming that our technology has no negative effect on DNA functionality.



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