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Isolation Methods of Peripheral Blood Mononuclear Cells in Spanish Biobanks: An Overview

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The Spanish Hematic Derivatives Group, consisting of 26 biobanks, was established in 2011. We describe here the viability results of our publically available standard operating procedure to freeze and thaw peripheral blood mononuclear cells (PBMCs). Our protocol maximizes PBMC viability while avoiding where possible inter-biobank and intrabiobank assay variability.

Keywords: PBMCs, SOP, biobanks, cryopreservation, functionality

Introduction

Cryopreservation enables peripheral blood mononuclear cells (PBMCs) that have been obtained from large number of people at different clinics and time points to be biobanked for downstream research projects. Many researchers want to use cryopreserved PBMCs that have been collected in longitudinal studies at predetermined time points, and/or to perform assays only after a sufficient number of samples are available. Suboptimal cryopreservation must be avoided because it reduces PBMC viability and alters the phenotype and immunological responses of those cells that remain viable.[1,2] Therefore, the protocols by which PBMCs are initially isolated and then cryopreserved are crucial.[3] There are many preanalytical and processing factors that influence PBMC functional responses, including isolation/purification protocols, storage, shipment, thawing speed, and temperature fluctuations during cryopreservation. Thus, several researchers have concluded that phenotype verification, proliferation, and functional assays should be performed on fresh or indeed cryopreserved PBMCs.[1,4–6]

To maximize PBMC viability, it is absolutely essential to strictly adhere to the designated and optimized standard operating procedure (SOP). The viability of cryopreserved PBMCs has a significant effect on the results of functional assays; for example, PBMCs with greater than 70% viability can be used for different functional studies such as ELISpot, immunomagnetic cell separation, cytokine production, and flow cytometry.[3,4,7,8] In this study, we describe how the biobanks within the Spanish Hematic Derivatives Group (SHDG) collaborate to cryopreserve, store, and thaw PBMCs and how they collaborate to develop and implement their SOP.

Materials and Methods

The study variables

The characteristics of PBMC donors, the types of tubes, cryopreservation media, and freezing temperatures were studied. We selected these variables because they influence post-thaw PBMC viability and function.

Seven out of the 26 biobanks participated in the SHDG work with PBMCs. In each of these seven biobanks, the PBMC isolation procedure was performed in a biological safety cabinet, using sterile consumables in accordance with biosafety good practices and regulations. The handling of all biological samples and blood collections was also carried out in accordance with the policies and procedures of the biobank facilities. All patients and healthy donors provided informed consent. In all seven biobanks, PBMCs were processed within 4 hours of the blood being collected (the

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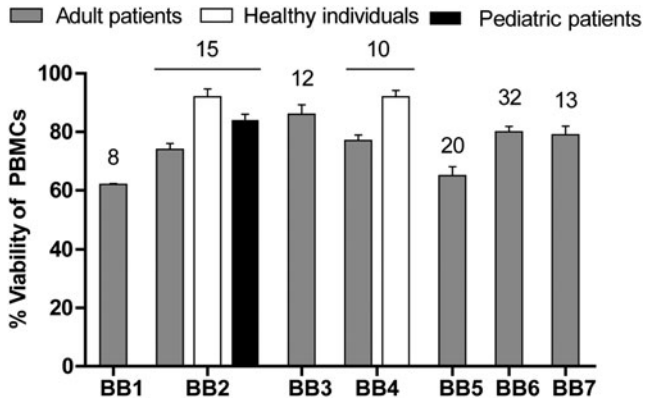

FIG. 1. Viability of PBMCs (Y-axis) and number of samples (number-top of each bar graph) from the seven biobanks. Data on PBMC viability have been reported as mean ± standard deviation of cell counts or percentages of viable cells (BB: biobank number). PBMCs, peripheral blood mononuclear cells.

biobanks are situated at the hospital site). The blood was kept at room temperature between its collection and processing.

Characteristics of the subjects

All seven biobanks cryopreserve PBMCs collected from adult subjects and one also collects samples from children. In addition to healthy adult volunteers, PBMCs are collected from patients with inflammatory, metabolic, infectious, and rare diseases; PBMCs are collected from hospital wards dedicated to oncology, endocrinology, allergy, and immunodeficiency.

Types of tubes

Erythrocytes, platelets, and granulocytes must be removed in a successful PBMC purification protocol, and the resultant PBMCs must maintain their functionality. The traditional method of PBMC purification is the Ficoll-Paque gradient centrifugation, often in BD Falcon™ conical tubes (Becton, Dickinson and Co.). The BD Falcon is an evacuated tube that contains an anticoagulant as well as the density gradient liquid that isolates the PBMCs by centrifugation. More recently, LeucoSep™ tubes (Greiner Bio-One) have been developed, which contain a porous membrane frit that prevents recontamination of the post-centrifuged PBMC fraction during harvest. Three of the seven biobanks collecting PBMCs use LeucoSep tubes. Overall, median post-thaw PBMC viability was 74.0% when prepared using BD Falcon conical tubes and 75.5% when using LeucoSep tubes, although the viabilities varied between the biobanks. Therefore, as already stated in the literature, we find using BD Falcon tubes to obtain PBMCs from whole blood is a viable alternative to LeucoSep tubes.[8–10] Our results show that BD Falcon and LeucoSep tubes return the same viability results ($p = 0.4768$). This indicates that all samples are acceptable for use in functional studies.

Results

Cryopreservation media

To prevent lethal ice crystal formation in frozen cells, 10% dimethyl sulfoxide (DMSO) is commonly added to fetal calf serum (FCS) to create the most frequently used cryopreservation solution for cells.[10–12] However, DMSO is toxic and its addition and removal are associated with a potentially detrimental osmotic shock to the cells.[13] Therefore, we compared the percentage of DMSO in the cryopreservation media used in the seven Spanish biobanks storing PBMCs. Each biobank always used the same cryopreservation media for all donors. The mean storage time for PBMCs was 14 months.

In adult patients, four of the seven biobanks (BB3, 4, 6, and 7 shown in Fig. 1) used 90% FCS + 10% DMSO as cryopreservation media, and their post-thaw PBMC viabilities were 77%–86% in adult patients (Fig. 1; Table 1). One out of the seven biobanks (BB2) used 92.5% FCS + 7.5% DMSO and had a slightly lower viability in adult patients (74%). However, BB2 also cryopreserved PBMCs from children using the same media, and these had higher viability (84%), so we do not believe that the slightly lower viability in adult patients in BB2 relates to the lower DMSO

Table 1. Type of Tubes, Media of Cryopreservation, Type of Anticoagulant, Viability Assesses

Biobanks	*Type of tubes*	*Media for cryopreservation*	*Type of anticoagulant*	*Viability assesses*
BB1	BD Falcon™	RPMI + 40% FCS + 10% DMSO	Heparin	Trypan Blue and Neubauer chamber. Manual counting in optical microscope
BB2	BD Falcon	FCS + 7.5% DMSO	EDTA	Trypan Blue and Neubauer chamber. Manual counting in optical microscope
BB3	BD Falcon	FCS + 10% DMSO	Sodic citrate	Trypan Blue and Neubauer chamber. Manual counting in optical microscope
BB4	Leucosep™	FCS + 10% DMSO	Heparin	Trypan Blue and Neubauer chamber. Manual counting in optical microscope
BB5	Leucosep	RPMI + 40% FCS + 10% DMSO	EDTA	ADAM-MC Automatic Cell Counting System
BB6	Leucosep	FCS + 10% DMSO	Heparin	Trypan Blue and Invitrogen™ Countess™ II FL Automated Cell Counter
BB7	Leucosep	FCS + 10% DMSO	ACD	Trypan Blue and Neubauer chamber. Manual counting in optical microscope

ACD, anticoagulant citrate dextrose; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; FCS, fetal calf serum; RPMI, Roswell Park Memorial Institute, a medium used in tissue and cell cultures.

concentration. The remaining biobanks (BB1 and 5) used 50% RPMI + 40% FCS + 10% DMS0, and these had lower PBMC viabilities of 62% and 65%, respectively. The lower PBMC viability in the biobanks using 40% FCS compared with the biobanks using 90%–92.5% FCS was statistically significant ($p < 0.0001$). Post-thaw PBMC viability was highest in the healthy adults (92% in each of the two biobanks collecting them). The difference in PBMC viability between the healthy and diseased adults was statistically significant ($p = 0.0022$). Reducing the percentage DMSO from 10% to 7.5% did not statistically significantly alter viability in healthy adults. None of the biobanks added antibiotics or antifungals to their cryopreservation media. All PBMCs were collected in hermetically sealed tubes, and as storage is in thermal dry nitrogen freezers, there is no liquid in contact with the vials. Microbial or fungal contamination of PBMCs has never been recorded.

Discussion

The biobanks pretested every new FCS batch for mitogenic and immune-modulating properties to ensure consistency. In view of its toxicity, it is logical to reduce DMSO concentration in cryopreservation media if possible, provided cell viability is not reduced as a result.[13,14] It has been previously published that hydroxyethyl starch (HES) can be used to partially substitute for DMSO, with a 6% HES plus 5% DMSO solution showing a very slight increase in PBMCs' recovery despite the reduction in the (toxic) DMSO concentration.[15] We obtained >70% post-thaw viability in PBMCs in four out of the six Spanish biobanks that used cryopreservation media with 10% DMSO and in the single biobank that used media with 7.5% DMSO. To introduce a common SOP in the seven Spanish biobanks, we selected the lowest percentage of DMSO to prepare the freezing media for PMBC.

Cryopreservation temperatures

Maintaining a stable and optimal temperature is crucial, especially during long-term storage. PBMC viability increases with decreasing temperatures.[16] Several researchers have studied the influence of the storage temperature and storage time on cell viability and functionality.[17–21] Exposure of cryopreserved PBMCs to suboptimal sample storage conditions with repeated temperature fluctuations reduces sample quality. Previous studies have reported that PBMCs cryopreserved in liquid nitrogen are amenable to immunophenotype analysis, and that such conditions do not significantly affect the level of apoptosis.[2,22,23] In contrast, other researchers have shown a loss of T cell response to antigenic stimulation.[18] The seven Spanish biobanks use isothermal dry nitrogen freezers because they provide added user safety by eliminating any contact with or splashing of liquid nitrogen and by eliminating the temperature fluctuations. Maintenance of optimal storage conditions is also critical, and six out of the seven biobanks have constant alarm and monitoring systems. All seven SHDG biobanks use water baths at 37°C and resuspend the PBMCs in RPMI 1640 medium and 10% FCS to thaw the frozen vials of PBMCs.

Based on the data described here, the SHDG developed a consensus cryopreservation protocol (SOP available at www.redbiobancos.es). The SOP consists of sample collection, preparation in appropriate tubes, use of 92.5% FCS + 7.5% DMSO media for cryopreservation, and freezing and low temperature storage in isothermal dry nitrogen freezers without any temperature fluctuations, to avoid cell damage (www.redbiobancos.es).

Theoretical and practical courses are given to the biobank technicians to enable them to correctly implement the SOP. This training includes all the steps described in the SOP from the moment of receiving samples to freezing and thawing them. The PBMCs from the seven biobanks have been used in 58 research projects, 8 clinical trials, and 89 published articles.[24–34]

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Author Disclosure Statement

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