

## Quality Assurance Program for Peripheral Blood Mononuclear Cell Cryopreservation<sup>∇</sup>

Adriana Weinberg,<sup>1\*</sup> Raul Louzao,<sup>2</sup> Marisa M. Mussi-Pinhata,<sup>3</sup> Maria L. S. Cruz,<sup>4</sup> Jorge A. Pinto,<sup>5</sup> Maria F. Huff,<sup>6</sup> Andrea C. de Castro,<sup>7</sup> Maria C. Sucupira,<sup>8</sup> and Thomas N. Denny<sup>2</sup>

*University of Colorado Health Sciences Center, Denver, Colorado<sup>1</sup>; Duke University, Raleigh-Durham, North Carolina<sup>2</sup>; Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil<sup>3</sup>; Hospital do Servidor Estadual, Rio de Janeiro, Brazil<sup>4</sup>; Universidade de Minas Gerais, Belo Horizonte, MG, Brazil<sup>5</sup>; Universidade Federal de Rio de Janeiro, Rio de Janeiro, Brazil<sup>6</sup>; Hospital da Conceição, Porto Alegre, Rio Grande do Sul, Brazil<sup>7</sup>; and Universidade Federal de São Paulo, São Paulo, Brazil<sup>8</sup>*

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**Seven Brazilian sites participating in the Pediatric AIDS Clinical Trials Group international cryopreservation quality assurance pilot program cryopreserved and shipped peripheral blood mononuclear cells (PBMC) to a central U.S. laboratory for analysis. Cell viability and recovery significantly increased over time. A wet-laboratory training session conducted at the central laboratory significantly improved the quality of the cryopreserved PBMC.**

The utilization of cryopreserved peripheral blood mononuclear cells (PBMC) for immunologic assays has recently increased. These cells are particularly useful in clinical trials with low end-point frequencies, because they allow the performance of assays after the complete identification of the end points (1, 8). Cryopreserved PBMC can be assayed in batches, avoiding inter- and intralaboratory variabilities when they represent potential confounders (6).

The use of cryopreserved PBMC in immunological assays poses challenges, including the availability of adequate equipment and the need for technical proficiency. Assays must be adapted and validated for the use of cryopreserved PBMC, and the quality of the frozen cells has to be monitored to ensure reliable results in functional and phenotypic assays. We have previously shown that the results of functional assays are strictly dependent on the viability of the cryopreserved PBMC (9), such that <70% viability compromises lymphoproliferative responses to antigens and mitogens. PBMC with viability of  $\geq 70\%$  are also suitable for cytokine production studies, flow cytometric analyses, and immunomagnetic cell separation (4, 5, 7, 9). While cell recovery does not interfere with the results of immunologic assays, the recovery of only a small proportion of cells may preclude assay performance altogether. Based on these observations, cryopreservation quality assurance (QA) programs must monitor the viability and recovery of cryopreserved PBMC. We report here on an effort to assess and improve the ability of seven Pediatric AIDS Clinical Trials Group (PACTG) sites in Brazil to cryopreserve viable PBMC.

This program included seven Brazilian sites, the Division of AIDS Immunology QA (IQA) Program, and the PACTG Cryopreservation Working Group. Pertinent aspects of this study were approved by local institutional review boards and the Brazilian National Research Council. The PACTG

cryopreservation consensus protocol (<http://impaact.s-3.com/immmlab.htm>) was distributed to the sites and discussed in detail over conference calls. The first three QA rounds used PBMC from 5 to 10 healthy volunteers per site. Cryopreserved PBMC were stored in liquid nitrogen at the sites and shipped to the IQA on dry ice. The time elapsed during transportation and the amount of dry ice in the package upon arrival were recorded by the IQA. At the IQA, thawed PBMC were assessed for viability and recovery. Companion vials were also thawed at the sites to evaluate the effects of shipment on viability and recovery. Troubleshooting occurred with bi-monthly conference calls. After the first three shipments, technologists from five sites participated in a wet-laboratory workshop at the IQA for hands-on training. After the workshop, QA rounds included cells from human immunodeficiency virus (HIV)-infected and uninfected volunteers. A second wet-laboratory workshop was organized 1 year after the first one.

The viability of PBMC thawed according to the PACTG cryopreservation consensus protocol was measured manually by the trypan blue exclusion method. Statistical analysis (using Prism 4 software; GraphPad) of 11 QA rounds showed a significant increase over time of the cryopreserved PBMC viability, from a mean  $\pm$  standard error of the mean of  $61\% \pm 6\%$  to  $88\% \pm 2\%$  ( $P < 0.0001$ ; Fig. 1A). A significant increase in viability occurred after the first wet-laboratory workshop but not after the second one. The proportion of samples unsuitable for functional assays due to viability <70% decreased from 50% of 10 samples in the first QA round to none of 14 samples in the last round (Fig. 1B).

Recovery, defined as the number of viable thawed PBMC over the original number of cryopreserved cells, also increased over time, from  $71\% \pm 5\%$  to  $91\% \pm 9\%$  ( $P < 0.0001$ ; Fig. 2). The first wet-laboratory workshop caused a significant increase in recovery ( $P = 0.006$ ), but the second one did not ( $P = 0.70$ ).

Transportation time from Brazilian sites to the IQA was 48 h, with one exception when the package was held by U.S. customs officials. None of the poor viability or recovery of PBMC cryopreserved at the international sites could be as-

\* Corresponding author. Mailing address: 4200 E. 9th Ave., Campus Box C227, Denver, CO 80262. Phone: (303) 315-4624. Fax: (303) 315-1787. E-mail: [Adriana.Weinberg@uchsc.edu](mailto:Adriana.Weinberg@uchsc.edu).

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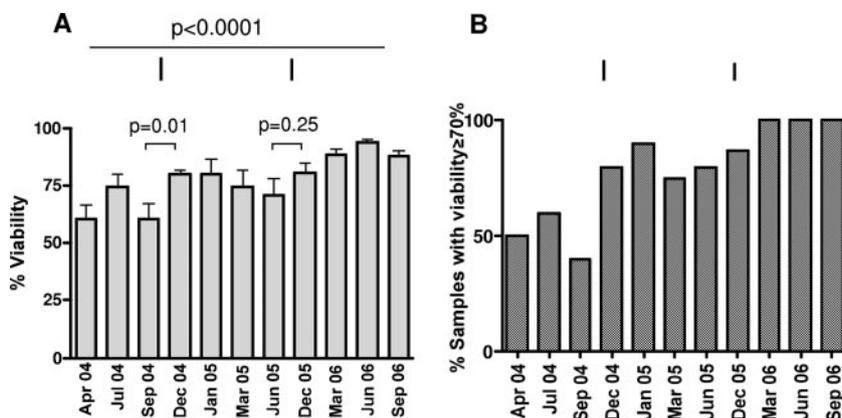


FIG. 1. Viability of PBMC cryopreserved at international sites. Bars in panel A represent means ± standard errors of the means of viability. Horizontal lines indicate linear trends over time and their corresponding *P* values. Vertical lines indicate the wet-laboratory workshops. Delimited lines and *P* values represent *t* test results comparing the viability immediately before with that after each workshop. Bars in panel B represent the proportion of samples with viability of ≥70% for each shipment. Vertical lines indicate workshops.

cribed to shipment conditions, because viability and recovery results were similar for paired cryopreserved samples thawed at the IQA site versus those on-site. All but one of the first shipments arrived at its destination with a satisfactory amount of dry ice. The cost of shipment from international sites averaged \$350/package.

Our data indicate that a proactive approach to PBMC cryopreservation, including wet-laboratory training and systematic conference call troubleshooting, was associated with fast and robust improvement of the quality of the cryopreserved PBMC. A cryopreservation QA program for U.S. ACTG sites has been in existence since November of 2000. Compared with the results obtained from U.S. sites (R. Louzao, personal communication), the baseline cryopreserved PBMC viability at the international sites was considerably lower, with an average of 77% versus 93%, respectively. However, the viability of the last QA round analyzed for this study was comparable to the viability registered for U.S. sites.

The single most important intervention that improved the

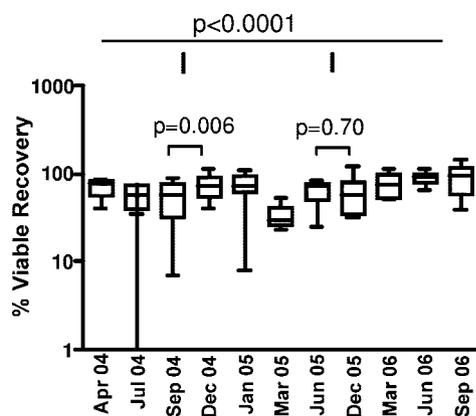


FIG. 2. Viable recovery of PBMC. Box-and-whisker plots show the median, quartiles, and range of the viable recovery at each time point. Horizontal lines and *P* values indicate linear trends over time. Vertical lines designate the wet-laboratory workshops. Delimited lines and *P* values represent *t* test results comparing the viability immediately before with that after each workshop.

quality of the cryopreserved PBMC was the first wet-laboratory workshop. The second workshop, which included different technologists from the same sites, was not associated with significant changes in the quality of the cryopreserved PBMC, suggesting that hands-on training of a single representative per laboratory may be sufficient to ensure that standardized procedures are adopted. The technical problems identified with the conference calls were laboratory specific, suggesting that individual counseling might be equally effective.

Sample shipment was satisfactory coming from international sites, and it did not affect the results of the QA program. However, international shipment was expensive, amounting to an approximately 10-fold value of domestic shipments.

This report shows the value of QA programs with respect to the quality of procedures, which is in accordance with that of previous studies (2, 3). QA programs may vary with respect to the degree of intervention, from a highly proactive approach, such as the one described here, to a passive approach, whereby samples are assessed and results are reported without requiring corrective actions. Although a proactive approach has the advantage of ensuring rapid and robust improvement of the quality of the cryopreserved samples, it has disadvantages that need to be weighed when designing a QA program. These disadvantages include higher cost and the ability to accommodate a limited number of participants in activities such as workshops and conference calls. The balance between the scientific and financial losses associated with samples that are poorly cryopreserved versus the higher cost of a proactive cryopreservation QA program may determine the ultimate choice of the approach.

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