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CD34⁺ Cell Isolation Comparisons with Frozen and Fresh Processed Cord Blood Units

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CD34⁺ stem cells are considered the key cellular subset in cord blood driving transplant engraftment, but they can also serve as starting source material for various cell types being developed as therapeutics or for use in research studies. Achieving clinically significant cell numbers from cord blood has been difficult without *in vitro* expansion, and optimizing the isolation of these cells for these efforts is a key need for the transplantation and cell therapy community. The isolation of CD34⁺/CD45⁺ cells from fresh cord blood units (CBUs) has been well documented. At the San Diego Blood Bank we assessed the isolation of CD34⁺ cells from processed CBUs that were held in cryostorage for times ranging from one to eighteen years or obtained as fresh unfrozen samples. All CBUs used were processed by ficoll enrichment prior to CD34 isolation and analyses. CBUs were assessed for TNC, CD34 count and viability by FACS analyses prior to, and after CD34 isolation. Only frozen CBUs having a viability >50% were included in the analyses. The Stemcell Technologies Robosep system was used for CD34 isolation under these different conditions and the parameters assessed were % yield, purity and viability. CD34 isolation from fresh CBUs demonstrated a CD34⁺ cell yield of 95%, with 83% purity and a viability of 97%, consistent with previously published data. When CD34⁺ cells were isolated from frozen CBUs, the % CD34⁺ cell yield ranged from 65 to 71%, purities ranged from 32 to 54%, and viability ranged from 77 to 84%. Results of these studies showed that viable CD34 enrichments were achieved with fresh and frozen CBUs that were stored for up to 18 years. In conclusion, while fresh and frozen CBUs gave acceptable CD34⁺ cell yields, purity and viability, fresh CBUs were superior in these parameters. Future studies using frozen CBUs are planned for further optimization.