Cancer therapy is a complex and growing field of medicine. With the advent of adoptive immunotherapy, coupled with excellent clinical outcomes in CD19+ hematologic malignancies, CAR-T cell therapy has created intense interest within the medical and scientific communities. Although genetic-modification of immune cells is one of the most advanced therapies, the manufacturing process still relies on traditional cell processing techniques. We have previously developed a quick and easy method to remove DMSO from thawed apheresis products and have now coupled this technology with the selection of CD3+ T-cells using ThermoGenesis’ technology, Buoyancy-Activated Cell Sorting (X-BACSTM) process. DMSO-removal was successfully achieved using the X-WASH™ System. Healthy adult cryopreserved apheresis samples were thawed at 37°C and diluted with thaw-wash media (2.5% HSA, 5% Dextran, and DNase I in saline). The sample was transferred to an X-WASH Disposable Cartridge (DC). Post-centrifugation, waste media was removed using positive pressure and new media was added using controlled negative pressure. Washed cells were

Results

The DMSO removal from cryopreserved samples and CD3+ cell isolation was accomplished in two phases. Each phase was studied for recovery efficiency and cell viability.

Overall recovery was calculated by multiplying values obtained in X-WASH and X-BACS steps. A mean CD3+ cell recovery of 81% was observed (n = 3).

Conclusions

DMSO removal using the X-WASH System resulted in cell recoveries of greater than 90% with no significant loss of viability. A mean CD3+ cell recovery of 84% was accomplished using the X-BACS process with high purity (>94%). In brief, we have developed an efficient method for removing DMSO and isolating CD3+ T-cells from cryopreserved apheresis samples that can be used for CAR-T cell manufacturing.