Interference for CD3+ and CD19+ subset enrichment by immunomagnetic beads

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BACKGROUND

Chimerism analysis monitors graft function after allogeneic hematopoietic cell transplantation (alloHCT). Subset chimerism detects engraftment in different cell lineages with higher sensitivity compared to whole blood only. Diagnostic utility depends upon achieving sufficient purity of enrichments, as contamination by non-target cells affects the accuracy of lineage-specific analysis.

This case illustrates the effects of interferences on immunomagnetic-based cell separation and subset chimerism analysis.

CASE

A 29-year-old female with acute myeloid leukemia had undergone alloHCT. In July 2023, post-transplant peripheral blood (PB) was received for subset chimerism analysis. Purity and DNA yield of the enrichments were acceptable (purity >80%; DNA concentration >2ng/uL). Chimerism testing showed 100% donor in B and myeloid cells and mixed chimerism in T cells (39% donor). In the following months, however, T and B cell enrichments from subsequent PB had consistently low DNA yield, unacceptable purity, and ≥95% donor chimerism. [**FIGURE 1**]. Notably, the patient had multiple false positive serum pregnancy tests, suggesting immunoassay interference.

FIGURE 1

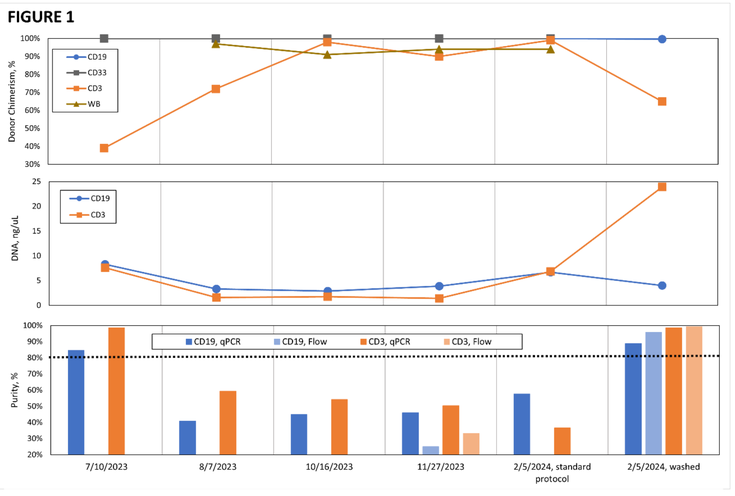

FIGURE 1: Chimerism results, DNA concentrations, and purity of enriched subsets for this patient from July 2023 through February 2024. Top, Donor chimerism (%) in whole blood (WB, triangle), T cell (CD3+, orange square), B cell (CD19+, circle), and myeloid cell (CD33+, grey square) subsets. Middle, Concentrations (ng/uL) of DNA extractions in T cell (CD3+, square), B cell (CD19+, circle). Bottom, Purity (%) of enriched subsets assessed by flow cytometry (CD19, light blue; CD3+, light orange) or qPCR (CD19+, dark blue; CD3+, dark orange).

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To investigate for potential interference, we developed a cell washing protocol [**FIGURE 2**], using manufacturer supplied buffer (1x RoboSep Buffer, containing PBS and FBS) for six washes, after which, CD3+ and CD19+ enrichments were performed using positive selection immunomagnetic beads (StemCell Technologies). Purity of these enrichments was assessed by flow cytometry and qPCR (Accumol). Chimerism testing was performed using an NGS based assay (Chimerism MD kit, Scisco-Genetics).

FIGURE 2

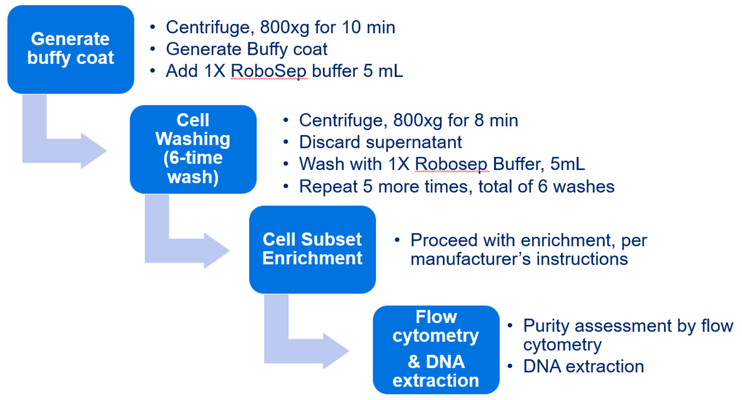

FIGURE 2: Cell Washing Protocol. Flow chart describing steps of cell washing protocol from peripheral blood through enrichment, flow cytometry, and DNA extraction.

Using the cell washing protocol, higher amounts of DNA were recovered for CD3+, but not CD19+. The purity was improved for CD3+ and CD19+, as assessed by both flow (99.3% CD3+ and 95.9% CD19+) and qPCR (98.6% CD3+ and 88.8% CD19+). [**FIGURE 3**]

Chimerism results were significantly different between subsets obtained by our standard enrichment method (CD3+ and CD19+ 100% donor) and by cell washing protocol (CD3+ 65% donor and CD19+ 99.6% donor) [**FIGURE 1**].

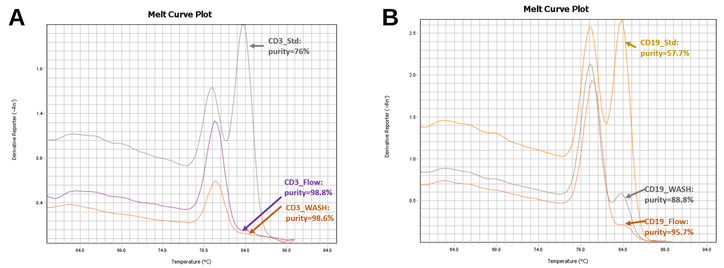

FIGURE 3: Purity of each subset enrichment was calculated by melting curve analysis from qPCR, which identified two peaks: first peak (*left side*) corresponds to the amplification of the Ultra-Conserved Sequence (UCS), and the second peak (*right side*) corresponds to the amplification of specific fragment of the non-T (**A**) or non-B population (**B**), corresponding to the domains excised during T or B cell differentiation.

Fluorescent activated cell sorting (FACS) was also performed in parallel to obtain CD19+ and CD3+ enrichments [**FIGURE 4**] as a comparison to cell-washing protocol. Chimerism results from flow-sorted CD3+ and CD19+ enrichments were consistent with chimerism results from cell washing protocol, *data not shown*.

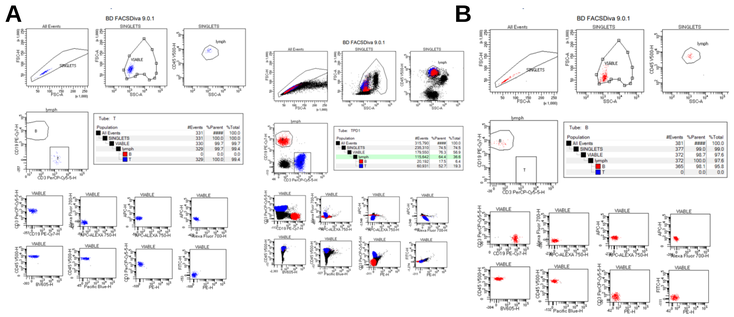

FIGURE 4: CD3+ (A) and CD19+ enrichments were obtained FACS. Scatter plots demonstrate high purity of T (*blue*) and B (*red*) cell enrichments.

CONCLUSION

Unexpected low recovery and purity of CD3+ and CD19+ cels by our standard enrichment method was likely due to an interferent in the patient plasma which can be reduced by cell washing. Concurrent false positive pregnancy tests suggest presence of heterophile antibody. We have developed a cell washing protocol to reduce interference in similar cases in the future.