CD34 Stem Cell Mimics as reliable controls for Cell Therapy: Addressing challenges in CD34 expression in Stem Cells and Lot-to-Lot Consistency

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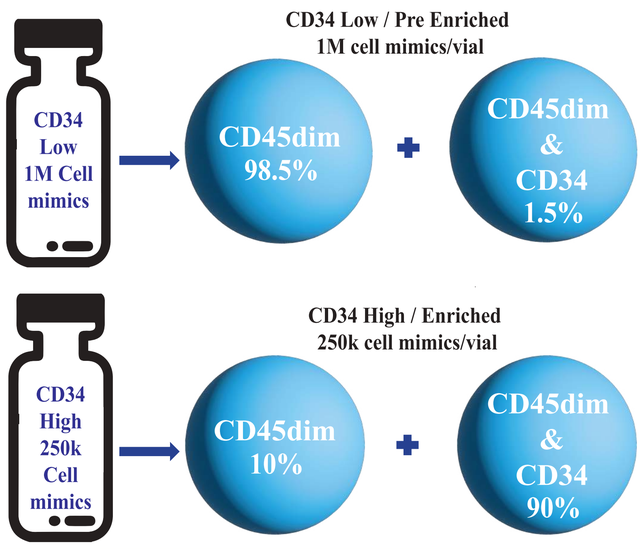
ABSTRACT

The use of CD34 stem cells in cell therapy is evolving, and ongoing research is continuously expanding our understanding of their potential applications and therapeutic benefits. Control cells are often needed throughout the cell therapy development process for phenotypic characterization of starting material or for quality control of downstream processing steps such as pre and post isolation. Commercially available stem cell controls are limited in their percentage of CD34 expressing cells, have inconsistent supply, and poor closed-vial shelf life stability requiring more frequent bridging studies. These commercial controls also require specific gating which may deviate from in-house protocols. The current alternative requires the use of mobilized peripheral blood in order to get high CD34 positive expression for use as a control which adds significant costs to the process. To address these challenges, Slingshot Biosciences has developed synthetic CD34 Stem cell mimics with CD45 dim+ CD34+ (90%) mixed with CD45 dim+ (10%) and CD45 dim+ CD34+ (1%) mixed with CD45 dim+ (99%). These can be complemented with cell mimics that contain a biologically relevant, heterogeneous mix of immune cell types, and therefore are a complete blood control without the drawbacks of donor-derived biologics. We show that these CD34+ stem cell mimics match the scatter optics of donor derived and commercially available controls on conventional and spectral flow cytometry instruments. These cell mimics also show superior lot-to-lot consistency compared to donor derived mobilized peripheral blood. This is the only non-biohazardous high CD34 expressing control that mimics biological samples such as mobilized blood, allowing confidence and standardization for downstream cell therapy manufacturing. These CD34 cell mimics are also represen-tative of pre and post CD34 enrichment samples without having to perform time-consuming enrichment processes. With our superior closed-vial stability, customers can secure a single lot for the entire duration of the study without worrying about inconsistent results or changing supplies.

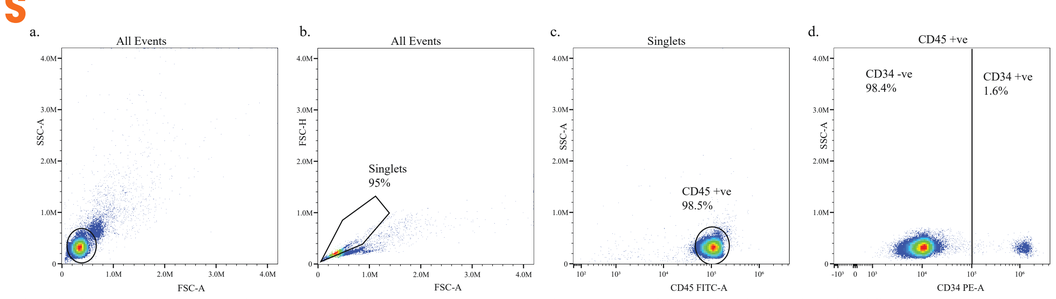
METHODOLOGY



- Prepare three lots of CD34 Low and CD34 High
- Analyze with CD45 antibody clone, 2D1 conjugated to FITC and CD34 antibody clone 8G12 conjugated with PE on the Cytek Aurora to evaluate inter-lot consistency in terms of % populations and MFI
- Analyze relevant biological samples to compare our product performance
- Analyze with other fluorophore conjugated antibody combinations to evaluate clone compatibility and instrument compatibility

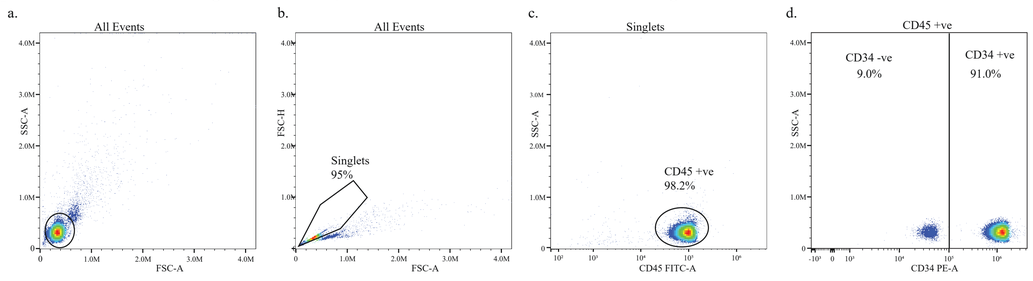
RESULTS

CD34 Low



Representative analysis of our CD34 low product on Cytek Aurora. a) Representative scatter of the cell mimics. b) Identification of singlets. c) Identification of CD45 positive cell mimics using 2D1 FITC. d) Identification of CD34 stem cells using 8G12 PE.

CD34 High

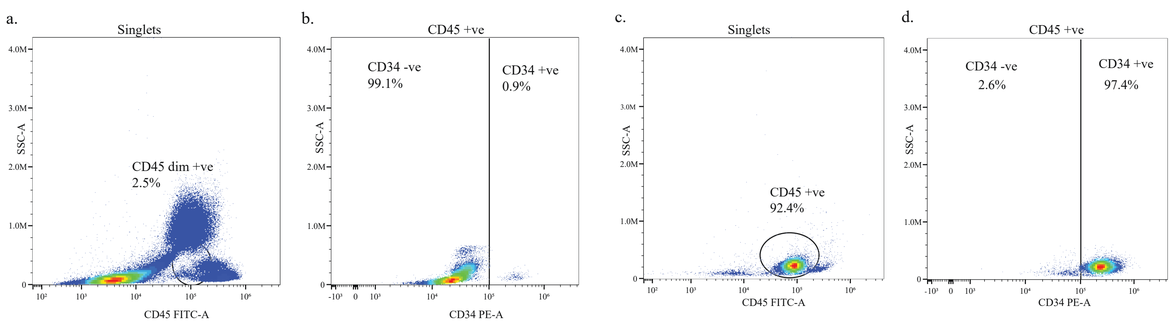


Representative analysis of our CD34 high product on Cytek Aurora. a) Representative scatter of the cell mimics. b) Identification of singlets. c) Identification of CD45 positive cell mimics using 2D1 FITC. d) Identification of CD34 stem cells using 8G12 PE.

Inter-Lot variability of % populations of three lots of CD34 Low and CD34 High. Analysis performed across three vials using flow cytometry. 2D1 FITC for CD45 and 8G12 PE for CD34 were used for staining

CD34 Low					
Percent Population	**A1**	**B1**	**C1**	**Average**	**%CV**
CD45	97.94%	98.22%	98.23%	98.13%	0.14%
CD34	1.65%	1.45%	1.61%	1.57%	5.53%

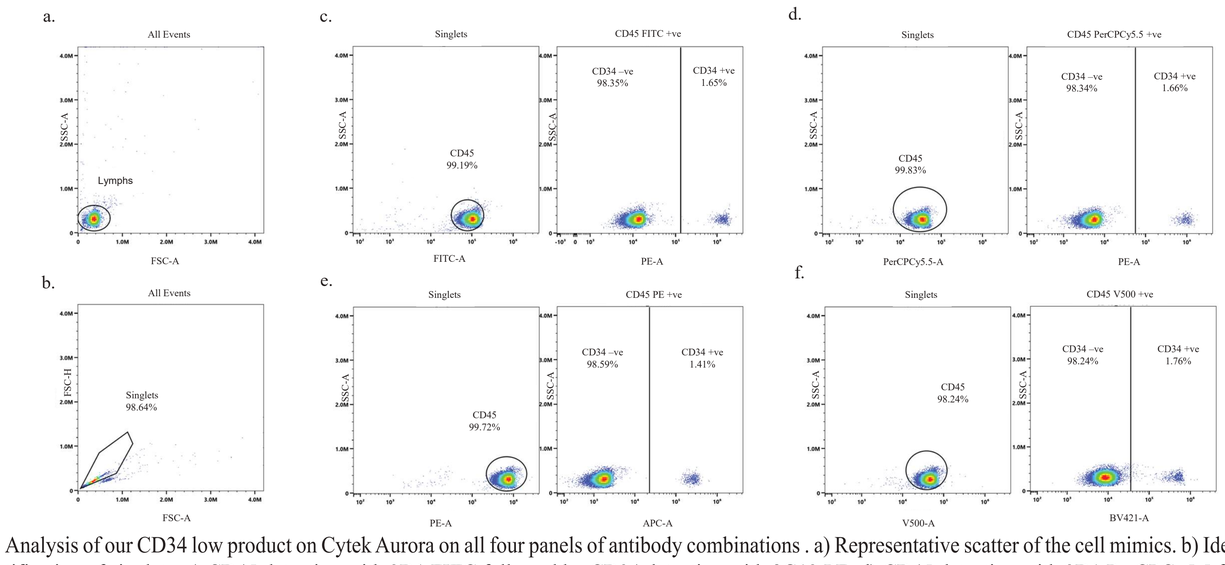
CD34 High					
Percent Population	**A2**	**B2**	**C2**	**Average**	**%CV**
CD45	9.08%	9.59%	8.44%	9.04%	5.20%
CD34	90.59%	90.12%	91.30%	90.67%	0.53%



Representative analysis of blood on Cytek Aurora using the gating scheme applied on our samples . Plot a is the identification of CD45 dim positive cells using 2D1 FITC. Plot b represents identification of CD34 stem cells using 8G12 PE. This represents how biological samples compare to our low product. Panels c and d represemt the analysis of immobilized leukopaks using the same antibody combination as whole blood and our product.This is how biological samples compare to our CD34 high product.

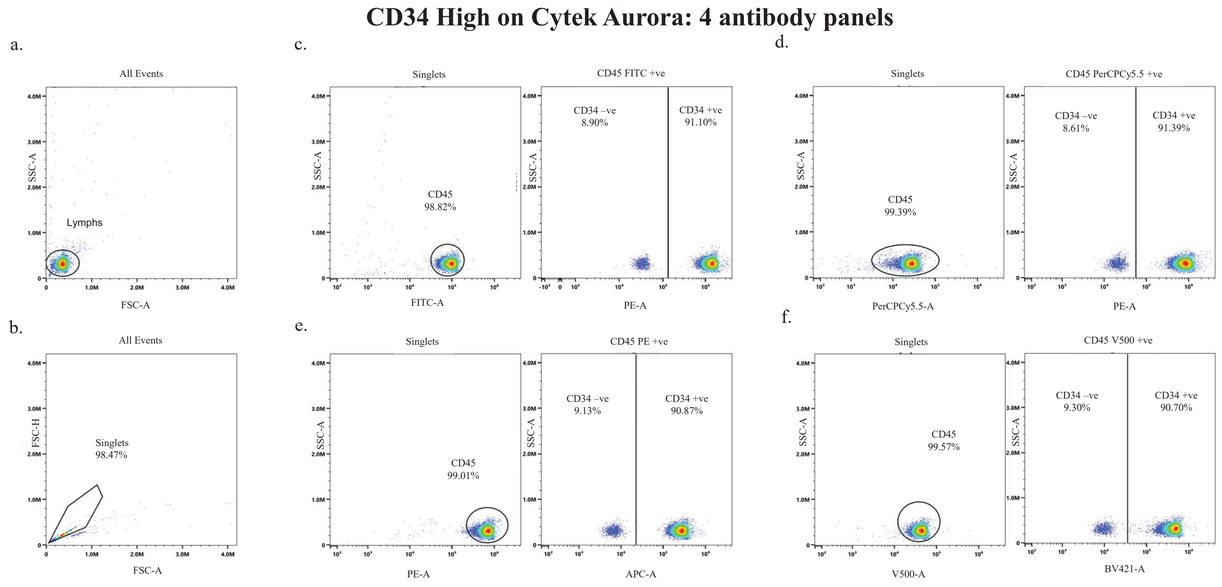
RESULTS

CD34 Low on Cytek Aurora: 4 antibody panels



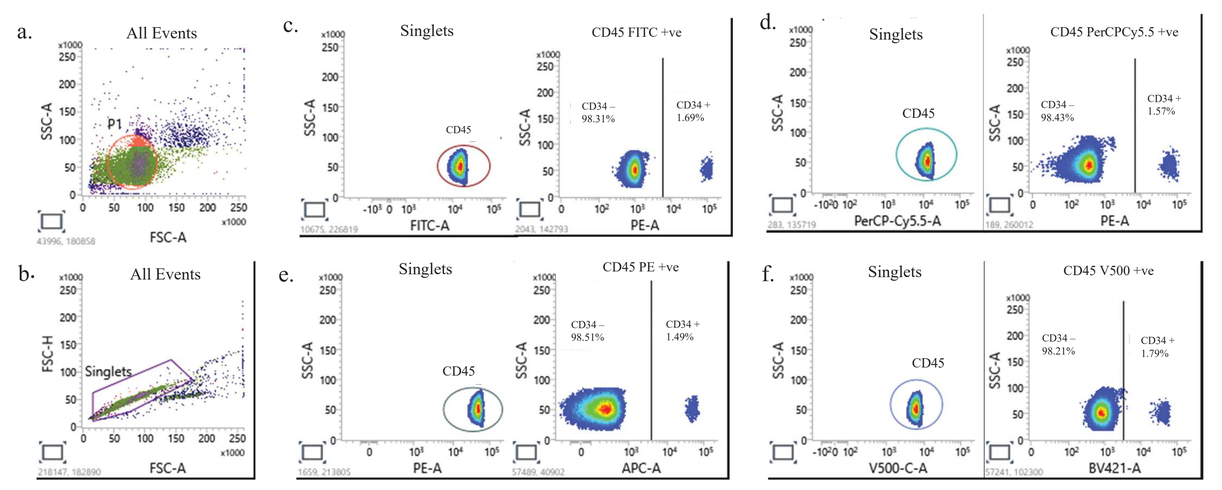
Analysis of our CD34 low product on Cytek Aurora on all four panels of antibody combinations . a) Representative scatter of the cell mimics. b) Identification of singlets. c) CD45 detection with 2D1 FITC followed by CD34 detection with 8G12 PE. d) CD45 detection with 2D1 PerCPCy5.5 followed by CD34 detection with AC136 PE.e) CD45 detection with MEM-28 PE followed by CD34 detection with 4H11 APC. f) CD45 detection with HI30 V500 followed by CD34 detection with 581 BV421.

CD34 High on Cytek Aurora: 4 antibody panels



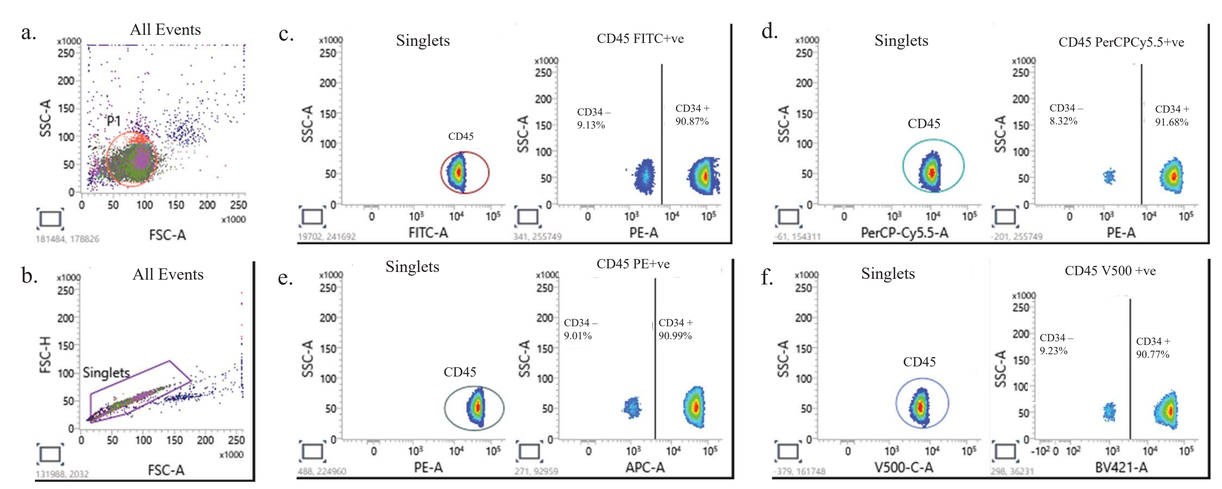
Analysis of our CD34 high product on Cytek Aurora on all four panels of antibody combinations . a) Representative scatter of the cell mimics. b) Identification of singlets. c) CD45 detection with 2D1 FITC followed by CD34 detection with 8G12 PE. d) CD45 detection with 2D1 PerCPCy5.5 followed by CD34 detection with AC136 PE.e) CD45 detection with MEM-28 PE followed by CD34 detection with 4H11 APC. f) CD45 detection with HI30 V500 followed by CD34 detection with 581 BV421.

CD34 Low on BD Lyric: 4 antibody panels



Analysis of our CD34 low product on BD Lyric on all four panels of antibody combinations . a) Representative scatter of the cell mimics. b) Identification of singlets. c) CD45 detection with 2D1 FITC followed by CD34 detection with 8G12 PE. d) CD45 detection with 2D1 PerCPCy5.5 followed by CD34 detection with AC136 PE.e) CD45 detection with MEM-28 PE followed by CD34 detection with 4H11 APC. f) CD45 detection with HI30 V500 followed by CD34 detection with 581 BV421.

CD34 High on BD Lyric: 4 antibody panels



Analysis of our CD34 high product on BD Lyric on all four panels of antibody combinations . a) Representative scatter of the cell mimics. b) Identification of singlets. c) CD45 detection with 2D1 FITC followed by CD34 detection with 8G12 PE. d) CD45 detection with 2D1 PerCPCy5.5 followed by CD34 detection with AC136 PE.e) CD45 detection with MEM-28 PE followed by CD34 detection with 4H11 APC. f) CD45 detection with HI30 V500 followed by CD34 detection with 581 BV421.

CONCLUSION

A robust, competitive CD34 based product has been successfully developed and shows superior and consistent performance compared to relevant biological samples. The CD34 low and CD34 high product is compatible with multiple fluorophores across three sets of antibodies for CD45 and four sets of antibodies for CD34. Intralot variability in MFI and % populations is <6%, and interlot variability between three lots in MFI and % populations is <6%. The TruCytes™ CD34 product is compatible with both conventional and spectral flow cytometers and is developed as a kit containing one vial of the CD34 low and one vial of the CD34 high sample, and serves as a positive reference control for stem cell therapies at both the pre and post enrichment stages.