Standards for Cell Therapy, Case Study: CD34+ Cell Enumeration Standards

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Outline

- Introduction to USP
- Need for standards in cell therapy
- Case Study: USP standards for CD34+ cell enumeration

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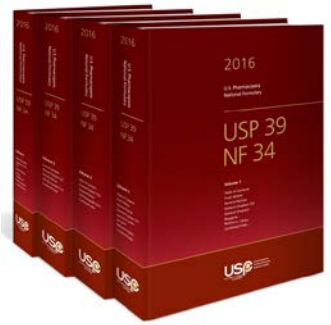
Introduction to USP

Ensuring Quality Medicines Throughout the Supply Chain

USP STANDARDS

- General Chapters
- Nomenclature and Labeling
- Monographs
- Reference Standards



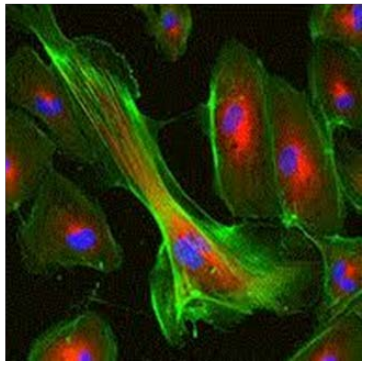
2 Need for Standards in Cell Therapy

Need For Standards in the Cell Therapy Field

- “Product consistency and lack of standards is possibly the single greatest challenge facing the field” (2016 Survey conducted by the Alliance for Regenerative Medicine)
- Reliable measurements for product characteristics to support informed decision making:
  - during development, manufacturing, and regulation.
- Lack of harmonized standards for cell therapy components, processes, or products
  - Lack of commonly accepted laboratory practices for cell therapy
  - Need for quantitative, validated, and robust assays for characterizing cell therapy products.
  - Need for reference materials to benchmark measurements and validation criteria for critical assays

Areas Where Standards are Needed

- Raw Materials: Consensus on acceptable raw and ancillary materials and their attributes
- Analytical and Testing Methodologies
  - Cell Counting: Improve consistency of methods used for cell counting
  - Cell Viability: Enhance methods and processes for determining and interpreting cell viability
- Product Quality and Characterization
  - Cell Characterization: Methods that utilize a cell's critical quality attributes
  - Potency Measurement: Standardized methods for measuring product potency
  - Residual Testing: Accurate and reliable methods



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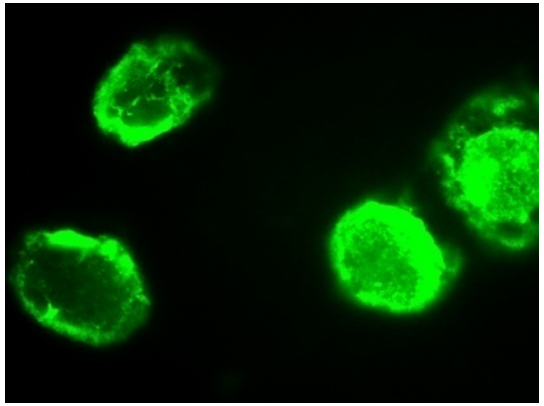
Case Study:
USP Standards for CD34+ Cell Enumeration

Case Study: USP Standard for Enumeration of CD34+ Cells

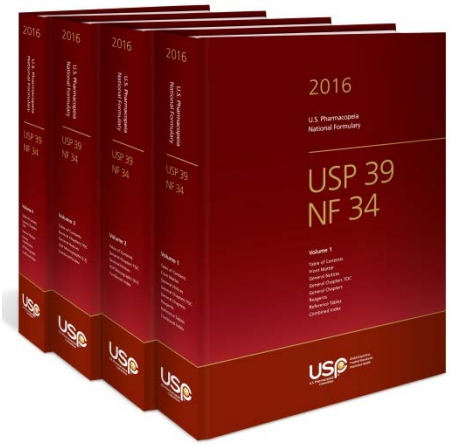
- CD34 Antigen expressed on the surface of almost all hematopoietic stem cells (HSCs)
- Enumeration of CD34+ cells used to determine yield of CD34+ cells mobilized in peripheral blood
- The number of CD34+ cells mobilized in hematopoietic grafts is a good predictor of success of apheresis and engraftment potential
- An accurate CD34+ cell count can help optimize the timing of apheresis collection
- Need for a standardized method for CD34+ enumeration to ensure consistent CD34+ counts in different laboratories and clinical centers.



Human CD34+ Cells

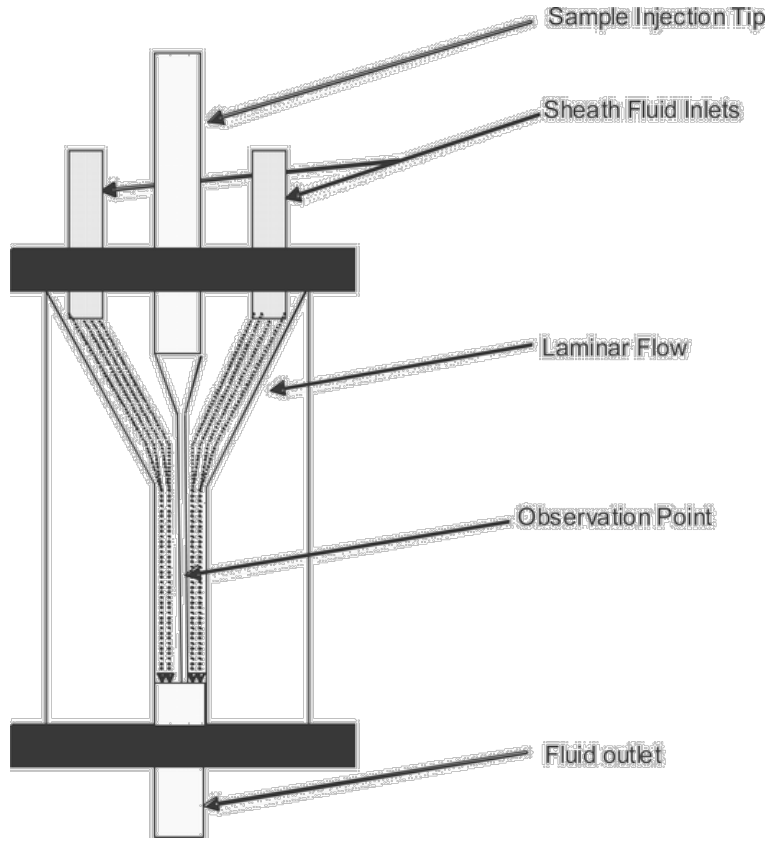
USP Chapter <127> *Flow Cytometric Enumeration of CD34+ Cells*

- CD34+ Cell enumeration is a rare event analysis, requiring specific gating instructions for detection
- Provides a standardized single platform, flow cytometric method for enumerating CD34+ cells
- The method can be used to quantitate CD34+ cells in samples of peripheral blood, leukapheresis products, bone marrow and cord blood
- Includes clear step by step instructions with figures for demonstrating sequential, Boolean gating based on the International Society of Hematotherapy and Graft Engineering (ISHAGE) protocol
- The USP CD34+ Cell Enumeration System Suitability Reference Standard is used to assess reagents and ensure correct gating for data acquisition and analysis



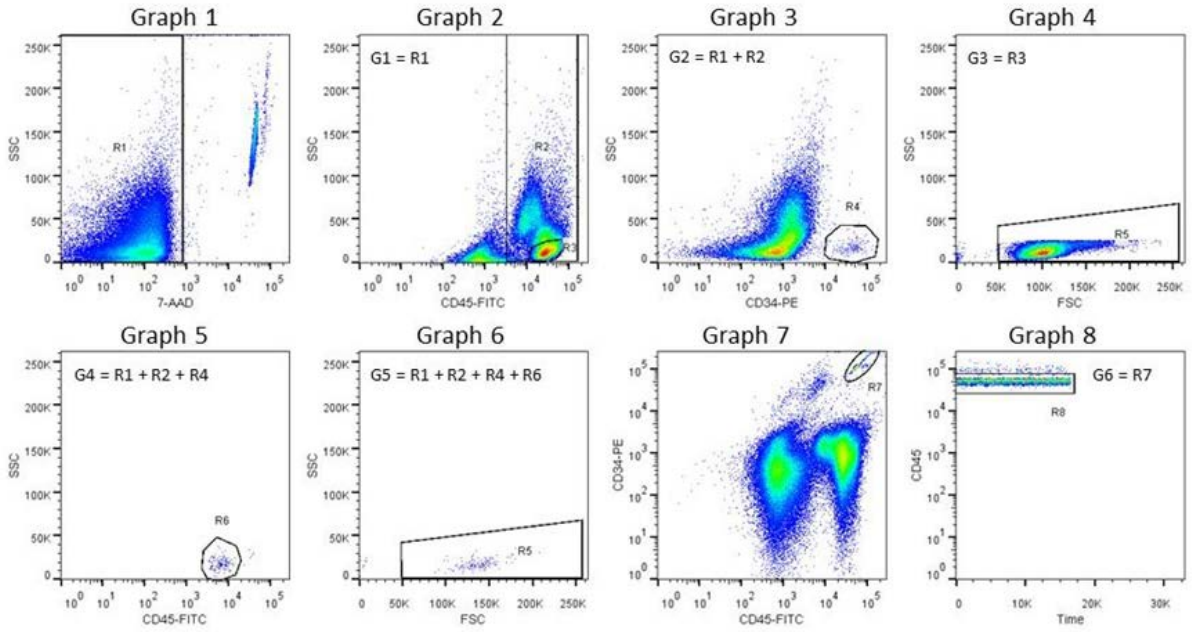
Enumeration of CD34+ Cells

- Flow cytometric enumeration of CD34+ cells
  - Most widely used clinical method to evaluate engraftment potential of peripheral stem cell collection
- CD34+ cell enumeration method relies on use of synthetic fluorescent microspheres (counting beads) as internal enumeration controls
- Homogenous counting beads are added to the sample at a known concentration and volume
- Wash steps are eliminated to avoid loss of beads
- After cells are stained and processed, the counting beads and cells are simultaneously analyzed on a flow cytometer
- The number of CD34+ cells/µL in the sample can be calculated by comparing the absolute number of target CD34+ cells and the number of counting beads detected in the same data file

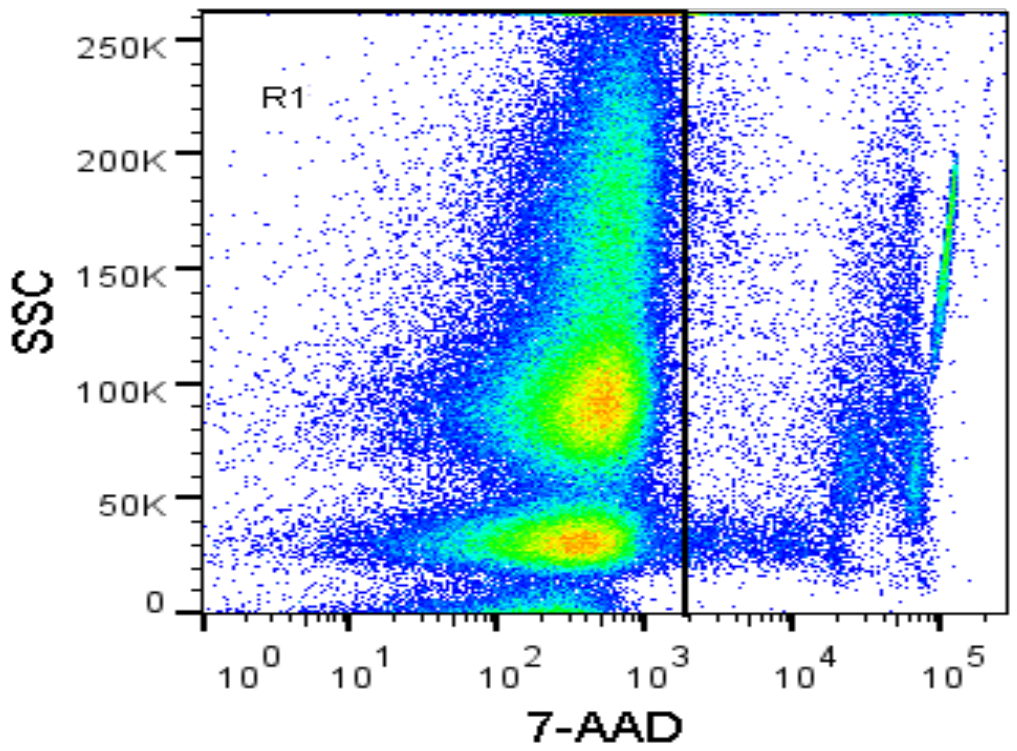


Identification of CD34+ Hematopoietic Stem Cells

- CD34+ Cell Enumeration is a rare event analysis requiring specific gating instructions for detection
- Cells are stained with fluorescently labeled antibodies against both the HSC antigen, CD34 and the pan-leukocyte antigen CD45
- CD34+ cells have light scattering characteristics similar to lymphocytes, expressing both CD34 and CD45, having dim CD45 expression and low SSC characteristics

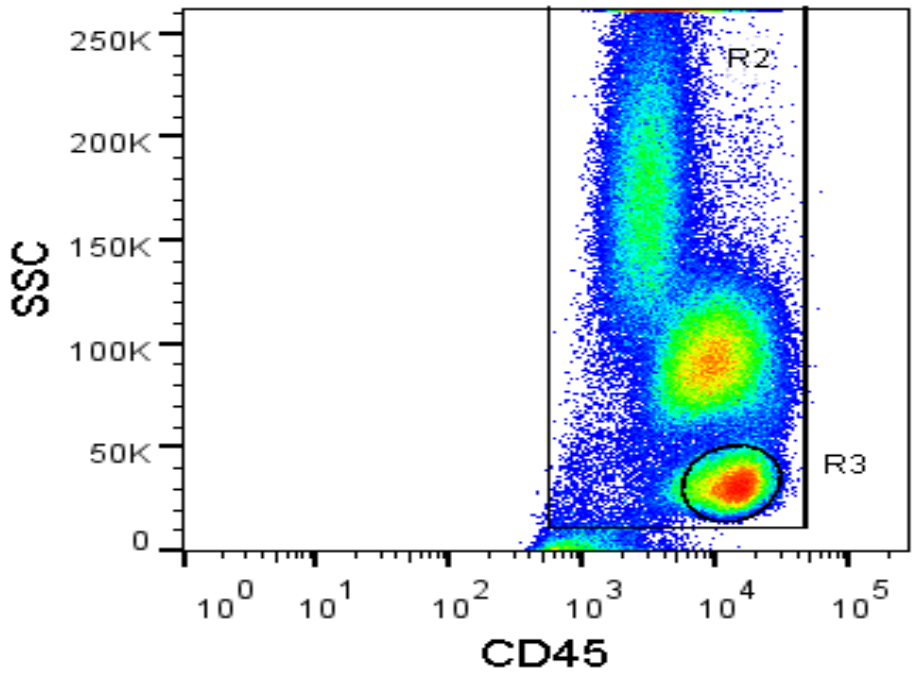


Step 1: Define Viable Cells



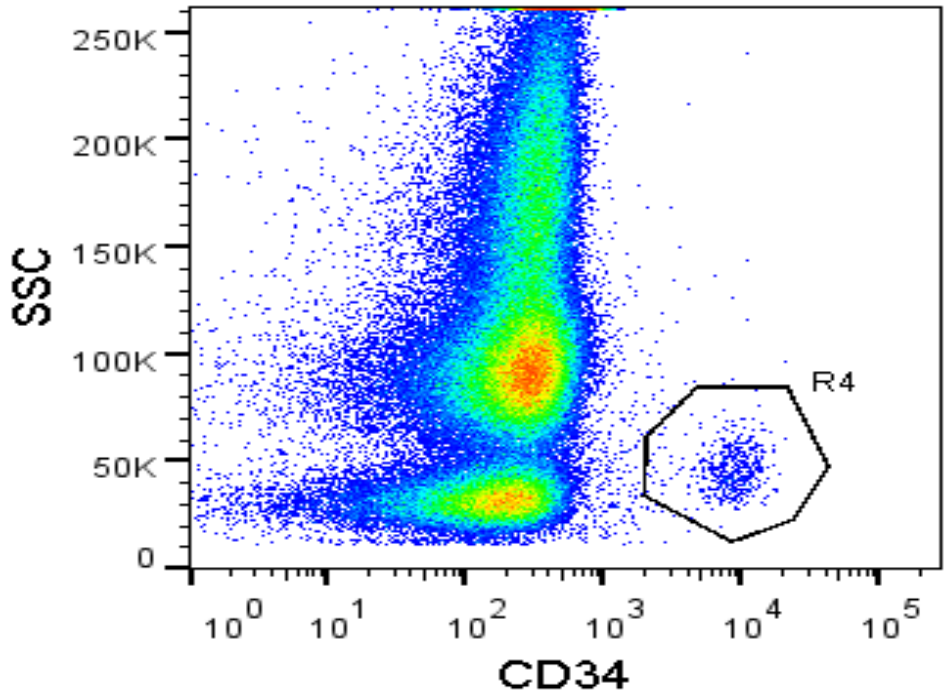
- Create Region R1 around events with little or no fluorescence.
- Viable cells and debris are included in the region, dead and dying cells and counting beads are excluded.

Step 2: Define Viable CD45+ Leukocytes and Lymphocytes



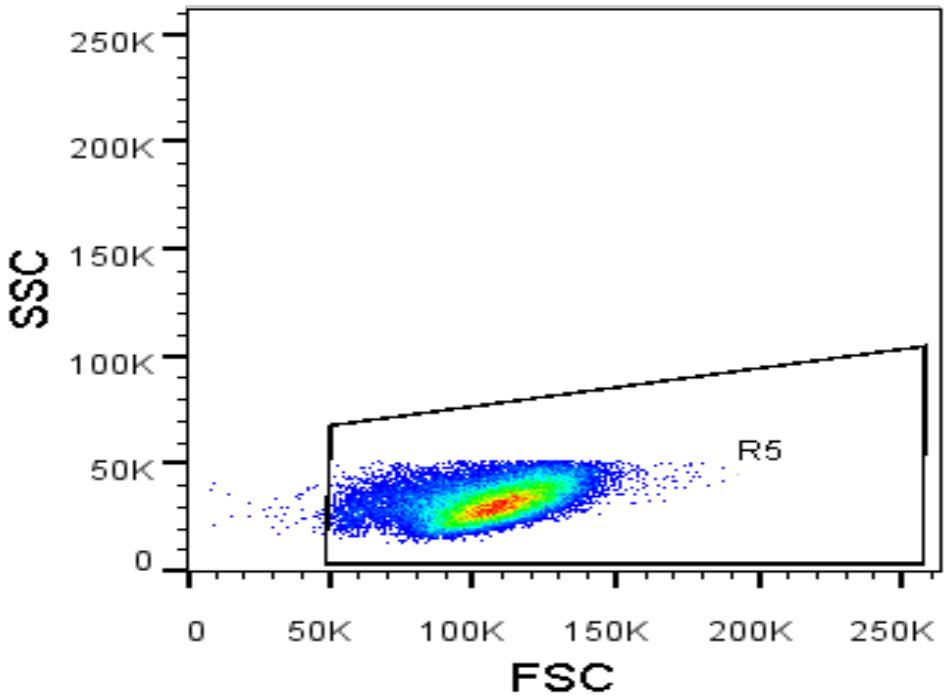
- Display R1 selected events. Create region R2 around events with CD45+ fluorescence, excluding debris.
- Within region R2, create region R3 around events with high CD45+ fluorescence and low SSC
- Region R2 includes CD45+ leukocytes, and R3 includes viable lymphocytes

Step 3: Define Viable CD34+ Cells



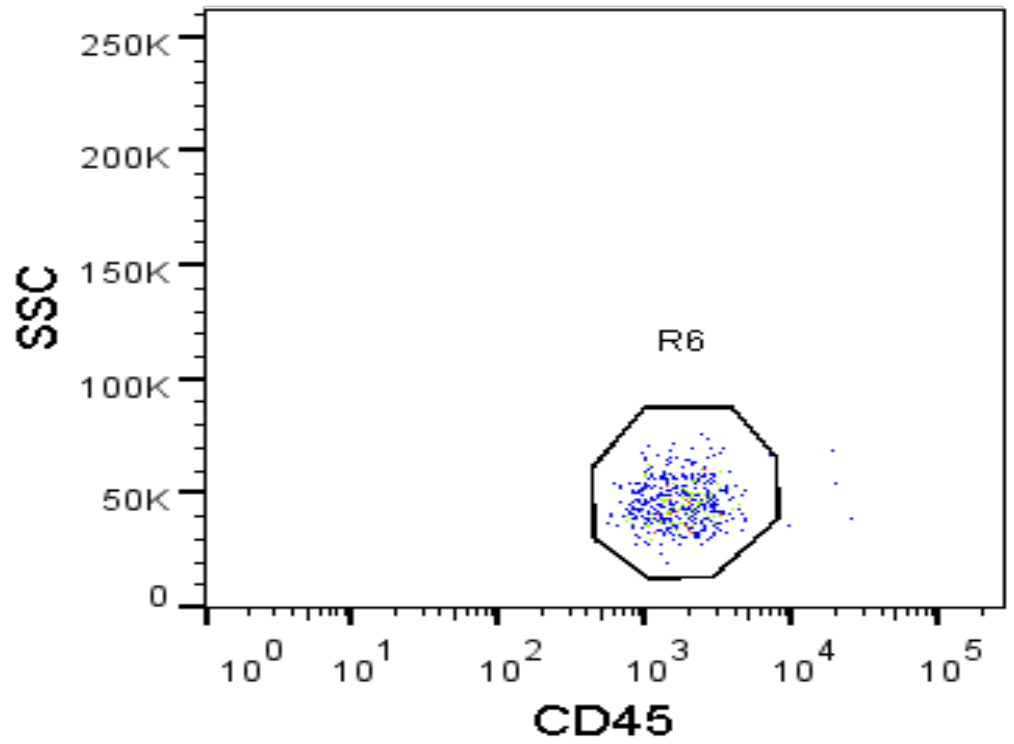
- Display R2 selected events. Create region R4 around events with CD34+ fluorescence and low SSC
- Region R4 contains viable CD34+ cells, other, CD34- cells are excluded

Step 4: Define Lymphocytes and Blasts



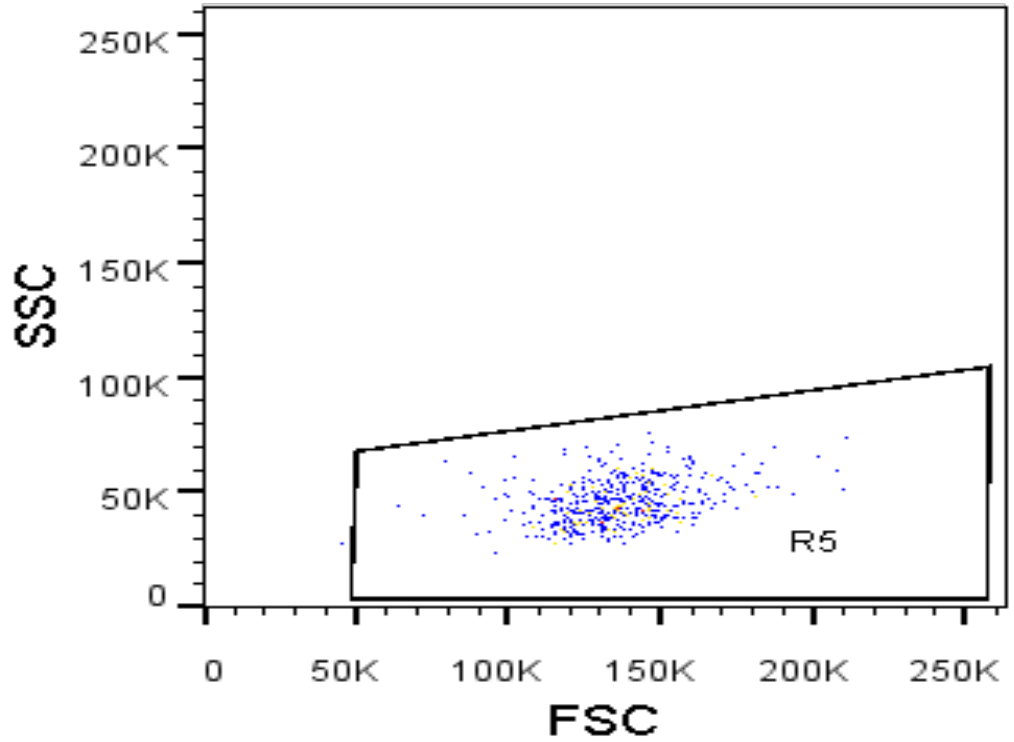
- Display R3 selected events. Create region R5 to exclude debris (low SSC and low FSC)
- Viable lymphocytes and blasts are included in the region, small debris and events with high SSC values are excluded.

Step 5: Define CD45 Dim, CD34+ Cells



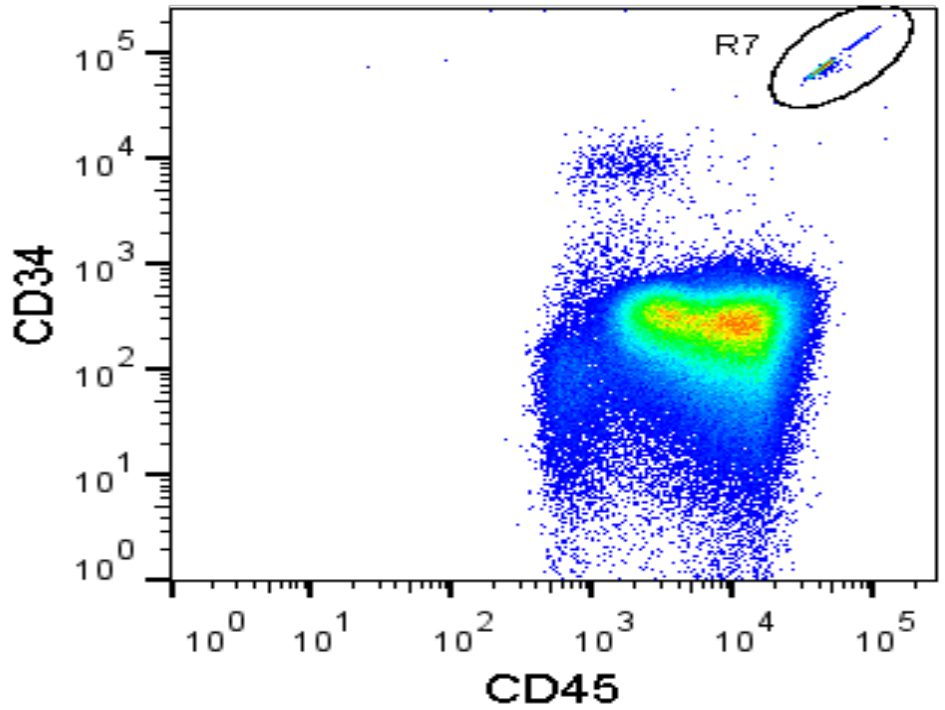
- Display R4 selected events
- Create region R6 around events with low-to-intermediate SSC and intermediate CD45 expression
- Viable CD34+ HSCs are included in the region

Step 6: Confirm CD34+ HSCs



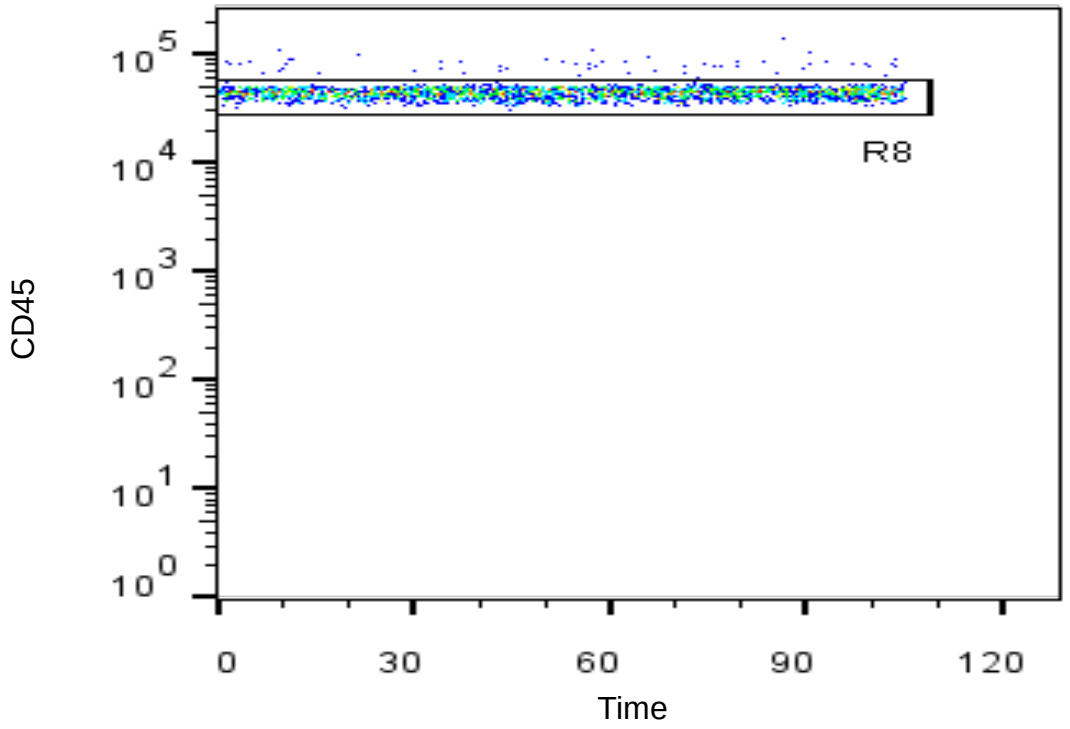
- Display R6 selected events, paste a copy of the R5 gate parameters into the plot
- Events in R5 have light scatter characteristics similar to lymphocytes and blasts, confirms identity of CD34+ HSCs
- Region R5 has confirmed viable CD34+ HSCs, debris and other irrelevant events excluded

Step 7: Quantify Total Counting Beads



- Display all ungated events
- Create region R7 around events with both high CD34+ and high CD45+ fluorescence
- Region R7 includes total counting beads, and excludes cells and debris

Step 8: Quantify “Singlet” Counting Beads



- When recommended by the counting bead manufacturer, display R7 selected events.
- Create region R8 around events with lower fluorescence intensity.
- Region R8 contains singlet counting beads and counting bead aggregates are excluded.

USP CD34+ Cell Enumeration System Suitability Reference Standard

- Made from mobilized peripheral blood collected by apheresis of a G-CSF mobilized donor.
- Contains human leukocytes, erythrocytes and CD34+ cells that have been fixed and lyophilized
- Reconstitutes in water and is ready to use. No lysis steps required.
- CD34 and CD45 staining comparable to fresh G-CSF mobilized peripheral blood cells
- Well-defined cell count and range assigned from a multi-laboratory collaborative study
- Used to assess the reagents used and ensure the correct gating during data acquisition for enumeration if CD34+ cells

Value Assignment for USP CD34+ Cell Enumeration System Suitability Reference Standard

- Multi-laboratory collaborative study established the mean and range for the number of CD34+ cell per vial
- Collaborators tested vials of candidate reference material using the method described in USP chapter <127> Flow Cytometric Enumeration of CD34+ Cells
- Analysis resulted in a label value of 1.24 x 10^4 cells per vial.
- After reconstitution in 500 µL of water, the mean concentration is 25 cells/µL with a range of 16-34 cells/µL
- Results should fall within the range provided in the USP CD34+ Cell Enumeration System Suitability RS certificate.

Summary

- USP general chapter <127> *Flow Cytometric Enumeration of CD34+ Cells* provides a standardized method for CD34+ cell enumeration.
- The USP CD34+ Cell System Suitability RS contains a known amount of CD34+ cells and can be used to assess reagents and ensure the correct gating for data acquisition and analysis.
- Standardizing the method for enumeration of CD34+ cells is important to ensure consistency of measurement in different laboratories and clinical centers.
- Pharmacopeial standards provide tools for the control of products development and testing strategies.
- USP is working on strategies to develop additional standards for cell and gene therapies.

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- Begins July 2018 for 2020-2025 cycle
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