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PEARLS OF LABORATORY MEDICINE

Enumeration of CD34+ Hematopoietic Progenitor Cells by Flow Cytometry

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CD34+ HPC by Flow Cytometry

- Reconstitute long-term multilineage hematopoiesis following marrow-ablative therapies
- Flow cytometry used to quickly determine potency of HPC products
  - Colony-forming assays take 10-14 days
  - Rare events masked by interfering populations
- 1995: ISHAGE developed simple method
  - Utilize CD34 antigen characteristic of HPC
  - 4-parameters (light scatter and 2-color immunofluorescence)
  - Sequential gating minimizes interference of nonspecific staining

CD34+ HPC by Flow Cytometry

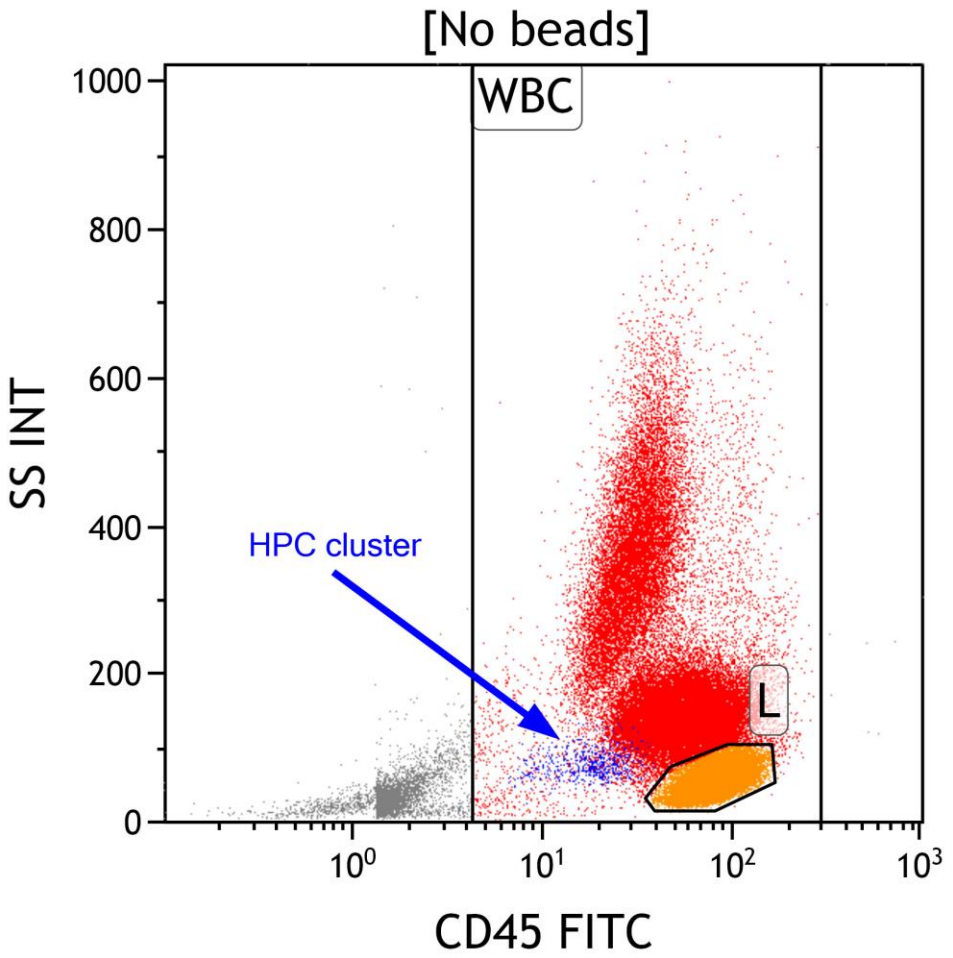
1) Express CD34 antigen
2) Express CD45 antigen like blasts
   - Positive but at low levels (i.e. $CD45_{dim}$)
3) Low side scatter
4) Low to intermediate forward scatter
   - Slightly higher than small lymphocytes

CD34+ HPC by Flow Cytometry

- Viability marker (e.g. 7-AAD) allows the exclusion of dead cells
- Use of fluorescent counting beads to calculate absolute concentration (single-platform)

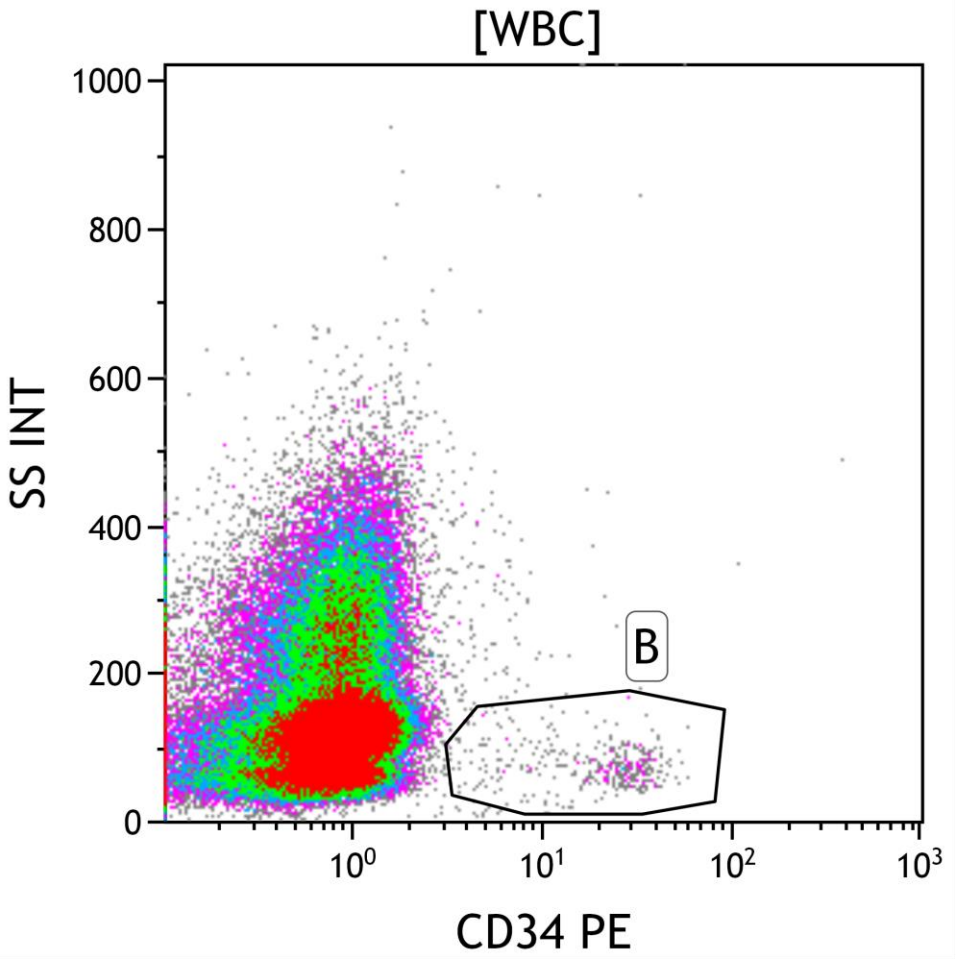
CD45 vs Side Scatter (Cellular events)

- Exclude contaminants such as platelets/aggregates, non-lysed RBCs and other debris
- Exclude CD45 negative events while taking care not to exclude HPCs which are $CD45_{dim}$
- Identify lymphocyte cluster (L) to be used later



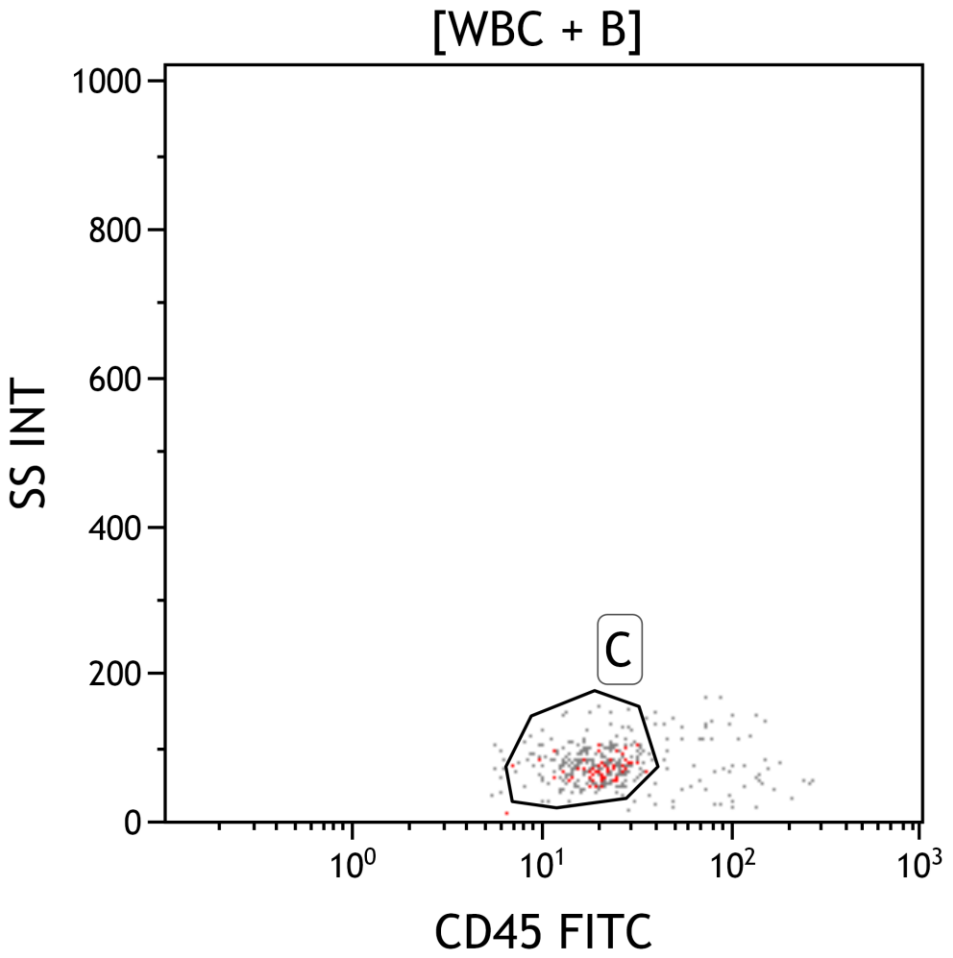
CD34 vs Side Scatter (WBC events)

- Used to include all potential CD34+ cells



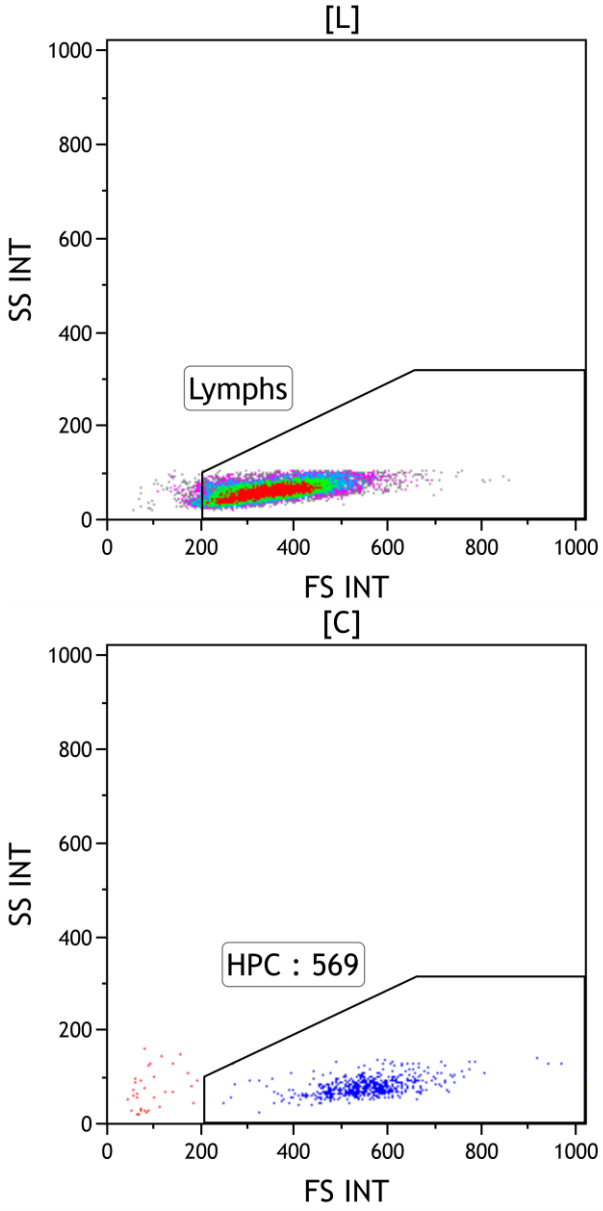
CD45 vs Side Scatter (CD34+ events)

- Identify the HPC cell cluster
- Lower CD45 expression than typical WBC
- Low Side Scatter, similar to Lymphocytes



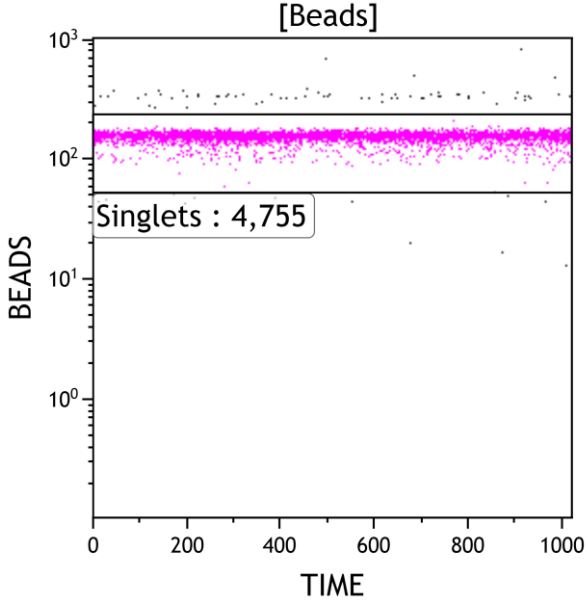
Forward vs Side Scatter (Top: Lymphocytes) (Bottom: CD34 cell cluster)

- Use Lymphocyte gate as a guide
- Mirrored HPC gate excludes platelets/aggregates that may stain positively CD45/CD34
- Events in this gate meet all 4 parameters to be classified as CD34+ Hematopoietic Progenitor Cells



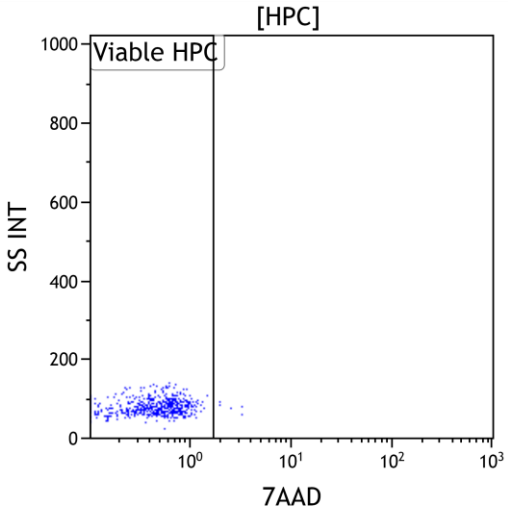
Time vs FL3 (Bead events)

- Select singlet bead events
- Ratio of bead to cell events used to calculate concentration



7-AAD vs Side Scatter (HPC events)

- Viable cells exclude the dye and appear negative for the marker



Gate	Number	%Gated
All	569	100.00
Viable HPC	564	99.12

Calculation of CD34+ HPC Concentration

$$\frac{\#\ of\ viable\ HPC\ events}{\#\ of\ singlet\ bead\ events} \times CAL\ factor$$

Example:

$$\frac{564\ (viable\ CD34\ cells) \times 1026\ (beads/uL)}{4{,}755\ (beads)}$$

$$= \mathbf{121.70\ viable\ CD34\ cells/uL}$$

NOTE: Multiply result by any dilution factor

Summary

- ISHAGE protocol is a single-platform flow assay that determines the absolute concentration of viable CD34+ HPCs in a sample or product
  - Calculation of HPC content in a product
  - Calculation of HPC dose per patient body weight

References

1. Sutherland D.R., Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. J Hematother 1996;5:213-26.
2. Sutherland D.R., Keeney M. Enumeration of CD34+ Cells by Flow Cytometry. Cellular Therapy: Principles, Methods and Regulations. The American Association of Blood Bankers (AABB) 2016:558-69.
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Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership**: No disclosures
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