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\textsubscript{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
Calculation of CD34+ HPC Concentration

\[
\frac{\text{# of viable HPC events}}{\text{# of singlet bead events}} \times \text{CAL factor}
\]

Example:

\[
\frac{564 \text{ (viable CD34 cells)}}{4,755 \text{ (beads)}} \times 1026 \text{ (beads/uL)} = 121.70 \text{ viable CD34 cells/uL}
\]

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


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