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Inter-site variability of CD34+ cell enumeration with flow cytometry analysis would be reduced by using newly developed microscopic cell counter

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Background

- **Flow cytometric analysis**
  - Standard method to enumerate CD34+ stem cell
  - Limitation
    - Less reproducibility among technicians and laboratories
    - Expensive instrumentation
    - High reagent costs
    - **Flow cytometric analysis**
- **Purpose**

To compare the CD34+ cell counts between data from 4 sites of flow cytometry and 2 sites of microscopic cell counter (ADAM II)

Materials & Methods

- 18 adult volunteer donors
- G-CSF mobilized peripheral bloods (PBs) and tube fragment of harvested peripheral blood stem cells (PBSCs) after G-CSF mobilization.
- Same ADAM II and 2 different institute
- 2 kinds of flow cytometry (FACS Calibur, FACS Canto II) at 4 different institutes

- First step: Assessment of correlation between the data (CD34+ cell counts) of ADAM II and flow cytometry
- Second step: Evaluation of the inter-site variability of CD34+ cell counts measured by flow cytometry and Adam II

Results

Correlation of CD34+ cell counts between FACS and ADAM II

The CD34+ counts obtained by Adam II showed high correlation with those of FACS Calibur and FACS Canto II.

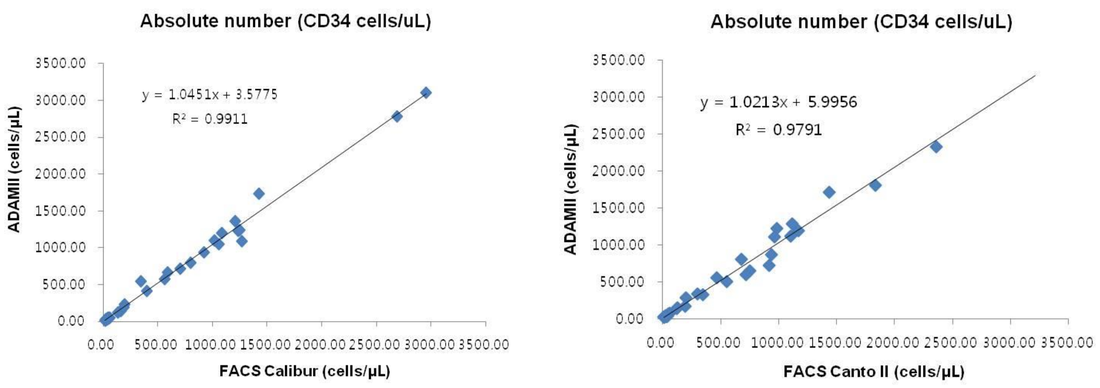

Fig. 1. The correlation for CD34 cell counts between the ADAM II and the FACSCalibur and FACS Canto II.

Reproducibility and dilution linearity of ADAM II

Four PBSC samples at CD34+ ells concentrations of 27.24, 52,56, 390.98 and 1373.23 cells/uL had CVs of 19.8%, 16.0%, 6.47%, and 7.2% respectively.

A dilution test of the ADAM II method demonstrated a linearity of r^2=0.99.

Table 1. Reproduction of the same sample tested 20 times

	Sample ID	Mean	Within Stain	
			SD	%CV
CD34 (/μL)	1	27.247	5.382	19.8
	2	52.559	8.418	16.0
	3	390.98	25.32	6.47
	4	1373.230	98.237	7.2

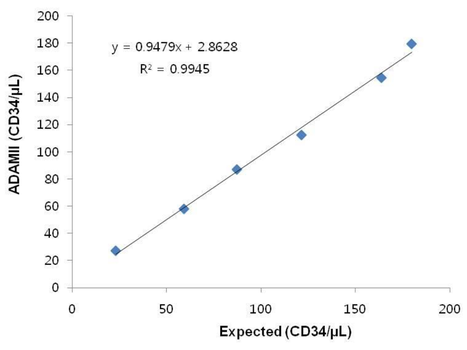

Figure 2. The correlation between CD34 counts as determined by the ADAM II and expected CD34 counts in diluted samples.

Inter-site variability of flow cytometry

The enumeration of CD34+ cells showed significant differences among 4 sites of flow cytometry (401.08±232.57, 1123.1±467.7, 1252.2±433.96, 340.2±270.87) ($p<0.001$)

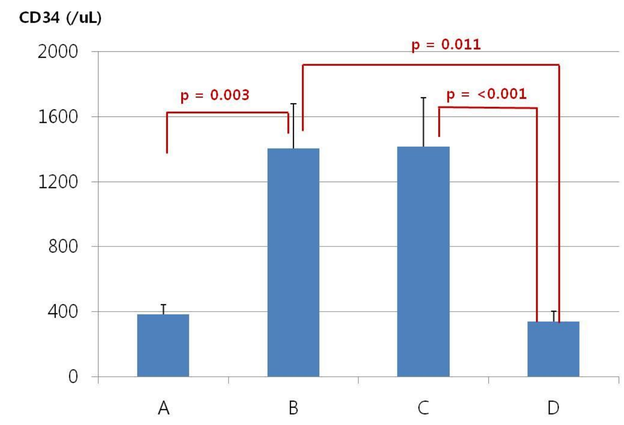

Figure 3. Total CD34 counts by FACS from 4 different institutes

Inter-site variability of ADAM II

There was no significant differences of TNC/CD34+ cell counts analyzed by ADAM II from 2 different institutes (B, C).

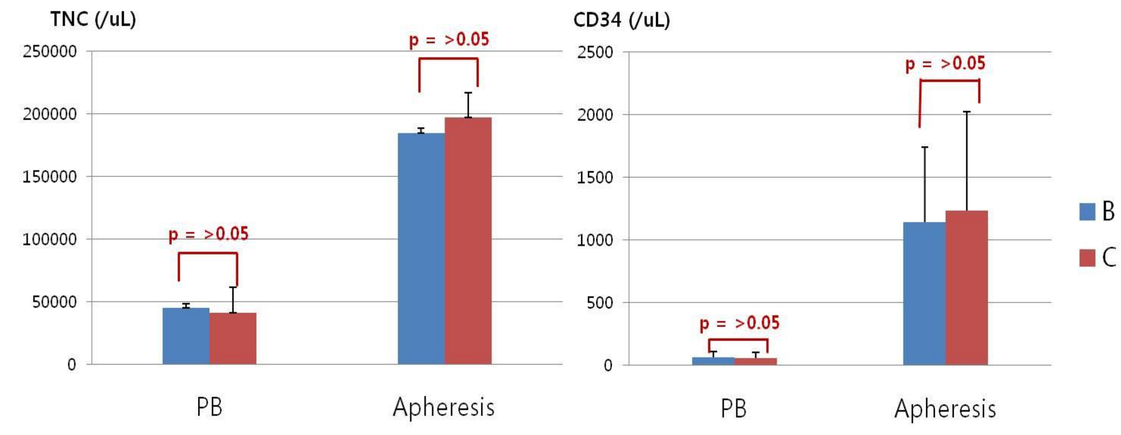

Figure 4. TNC/Total CD34 counts by ADAM II from 2 different institutes (B, C)

Conclusion

- **We observed the close correlation of CD34+ cell counts which measured by flow cytometry and Adam II. The dilution linearity and reproducibility of CD34+ cell counts by Adam II were demonstrated.**
- **The enumeration of TNCs and CD34+ cells by microscopic cell counter from 2 different sites showed consistent results, although the inter-site variability of CD34+ cell counts by flow cytometry was observed. Further studies for multi-site variabilities of ADAM II and FACS would be required.**