ADAMII™

New microscopic cell counter using autofocusing system does not require CD45 staining to enumerate CD34+ stem cells : comparison with flow cytometric analysis

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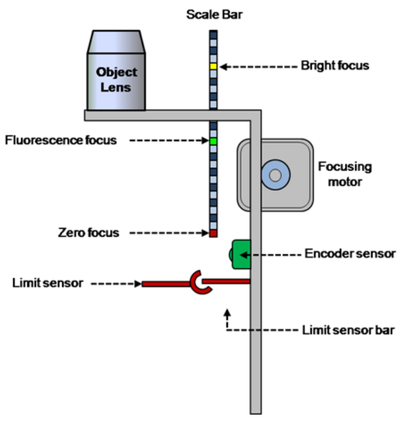
Autofocus principle



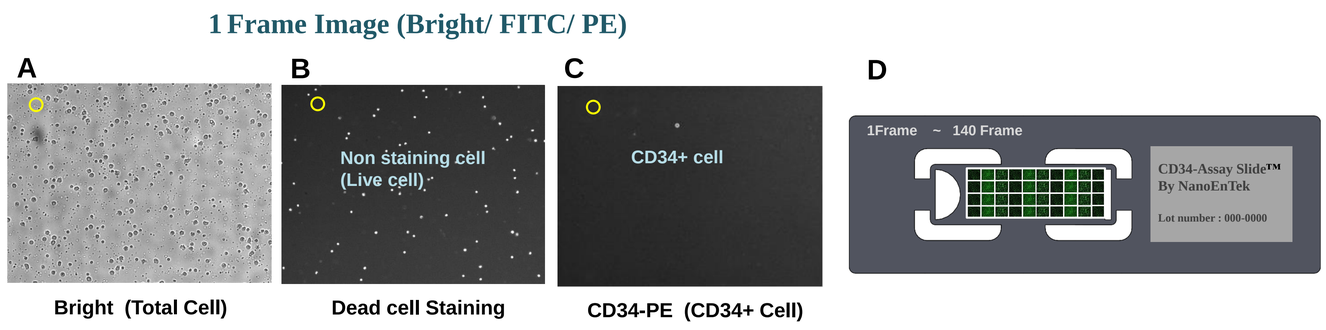
➢ The focus of bright or fluorescence image is aligned manually with a nobe in advance, then the z-asix movement distance (focus value) from the aligned position relative to the original stage position is calculated by Encoder sensor.

➢ Two or more different channels (bright, fluorescence) for an image position value is individually Measured and set in the system.

➢ Based on different established position values for each channel (bright, fluorescence), image focusing is done automatically and integrated image results are obtained

Principle of Viable CD34+ Cell Analysis

The ADAM™ simultaneously captures a series of bright field and fluorescent images of the sample in the CD34 Assay Slide™ and uses sophisticated digital image analysis algorithms to determine total and fluorescent cell counts and calculate their concentrations.



A) Bright - Total cells are counted and the cells and debris are discriminated by shape and size.

B) Dead cell staining (FITC) – Only dead cells are counted (Non staining cell : Live cell, Staining cell : Dead cell)

C) CD34 Ab staining (PE) - Only CD34+ cells are counted (Staining cell : CD34+ cell)

D) ADAMII assay slide - 140 Frame reading area

Introduction

Flow cytometric analysis has been a standard method to enumerate CD34+ stem cells in the field of hematopoietic stem cell transplantation. However, there are still some limitations such as expensive instrumentation, high reagent costs, and less reproducibility among technicians and laboratories in most cases. We compared the CD34+ cell data between flow cytometric analysis and the microscopic cell counter using a single stain with CD34 and autofocusing system which was recently obtained patent.

Method

1. Samples

- **Peripheral blood stem cell (PBSC) samples after G-CSF mobilization and G-CSF mobilized peripheral bloods from 18 adult volunteer donors.**
- **Each individual donor provided written informed consent prior to us obtaining the samples, and the study was approved by the Institutional Review Board of Hanyang University Hospital.**

2. ADAMII-CD34 Assay Procedure (Figure 1)

- **Sample were introduced into the ADAMII-CD34 Tube™ containing lyophilized fluorescence mixtures.**
- **RBC lysis buffer is added for the lysis of red blood cells.**
- **The reacted samples were loaded into the CD34 Assay Slide™ and read.**

3. Flow cytometry

- **FACSCalibur™ (BD Bioscience), FACSCantoII™ (BD Bioscience)**
- **Stem Cell Enumeration kit™ (BD Bioscience, Franklin Lakes, NJ)**

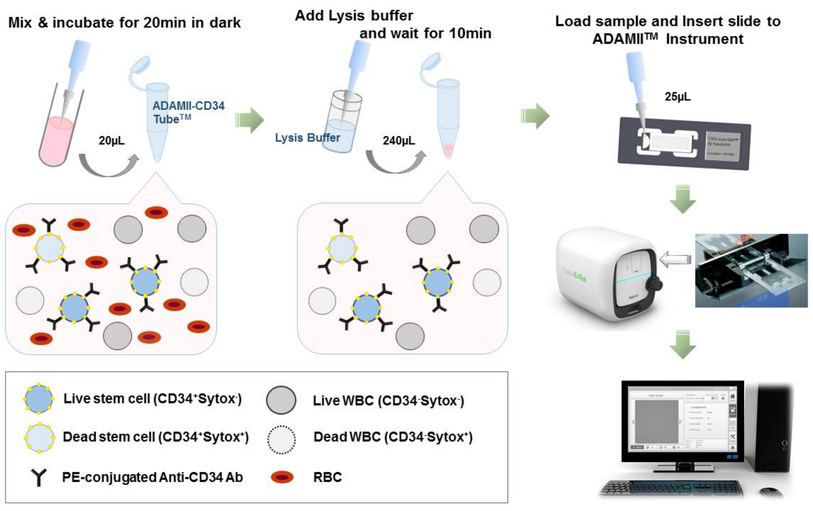


Figure 1. Description of ADAMII™-CD34 Counting Assay

Results

1. Method Comparison

Total 36 samples were used to compare ADAM II and flow cytometry (FACS Calibur and FACS Canto II). Linear regression analysis revealed a close correlation between the data for the absolute number of CD34+ and its cell fractions (%) obtained with the ADAMII™ and those obtained with the FACSCalibur™ for PBSCs (Absolute number of CD34, r2 = 0.99; CD34+ fractions (%), r2 = 0.98). Data obtained with the ADAMII™ and the FACSCantoII™ are presented below (Absolute number of CD34, r2 = 0.98; CD34+ fractions (%), r2 = 0.98).

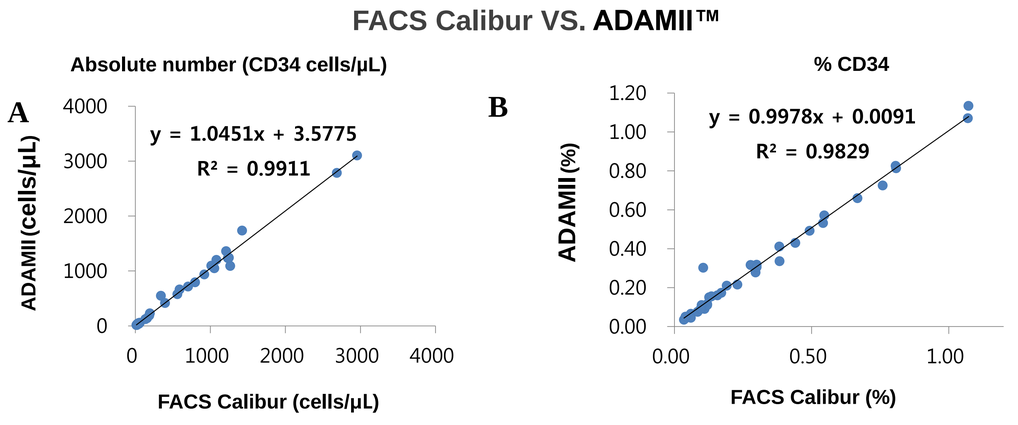


Figure 2. The correlation for CD34 cell counts between the ADAMII™ and the FACSCalibur™ A) Absolute number of CD34+ cells, B) Percentage of CD34+ cells

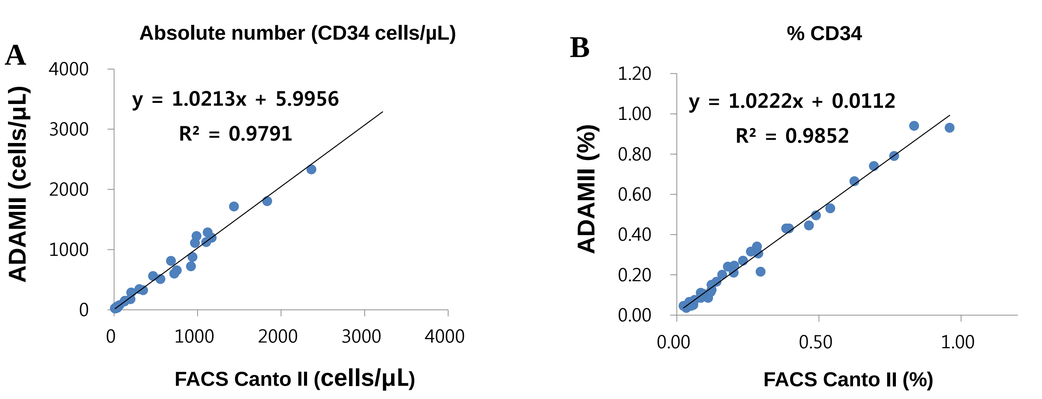


Figure 3 The correlation for CD34 cell counts between the ADAMII™and the FACSCantoII™ A) Absolute number of CD34+ cells, B) Percentage of CD34+ cells

2. Dilution Linearity

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

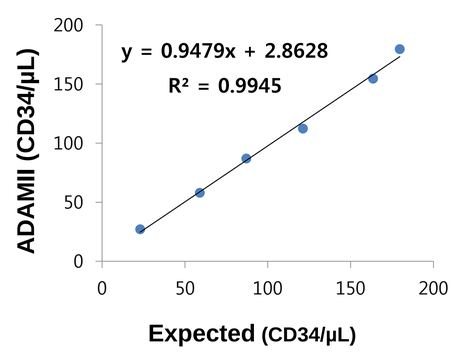


Figure 4 The correlation between CD34 counts as determined by the ADAMII™ and expected CD34 counts in diluted samples

3. FACS-CD45+ cells VS. ADAM II -total cells

The enumeration of CD45+ cells using flow cytometry were also comparable to the total cell counts in bright images of ADAM II.

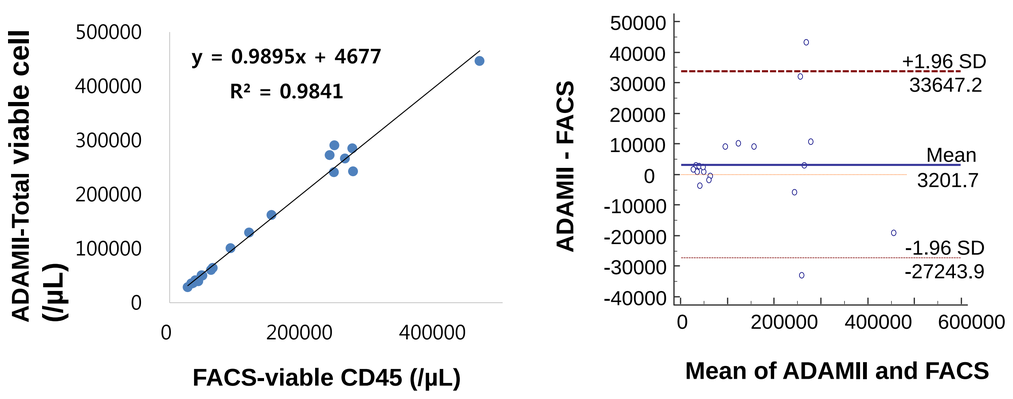


Figure 5 The correlation for Viable CD45 cell counts between the ADAMII™ and the FACS

4. Reproducibility

To assess reproducibility, some samples were counted 20 times, and coefficients of variations (CVs) were calculated.

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
CD34 (%)	1	0.066	0.014	20.4
	2	0.138	0.026	19.0
	3	0.32	0.02	7.73
	4	0.511	0.027	5.2

Conclusion

We obtained a high correlation of data between flow cytometry and ADAM II for enumeration of total/viable CD34+ cells, even without staining CD45 which is usually recommended for enumeration of CD34+ cells by flow cytometric analysis.

These results suggest that ADAM II CD34+ cell counting device can be potentially be applied in the field of stem cell assay with the advantages of reproducibility, accuracy, convenience, and inexpensiveness.