NanoEnTek

A PORTABLE MICROSCOPIC CELL COUNTER (ADAMII™-CD34) FOR ENUMERATING CD34+ CELLS

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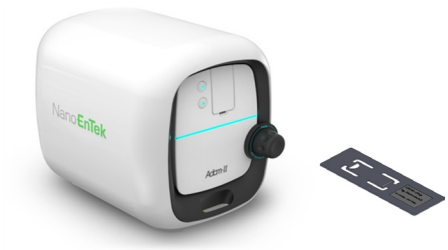
ADAMII™-CD34

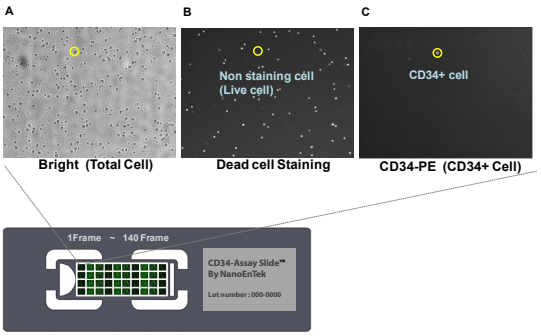


- 4-channel (Bright, Yellow, Green and Red) Light source
- User–friendly, bench top design for simple, fast, and highly accurate four-parameter population analysis
- Image analyzer to perform assays for CD34+ cells as well as cell viability (CD34, Total cell) simultaneously.
- Other routine cell analysis is possible by upgrade software
  - : CD3, CD4, CD8, CD45, CD33
  - : Apoptosis, Cell cycle
  - : rWBC, Anti D, CSF (RBC, WBC count)
  - : Quantitating GFP & RFP expressing cells
- Can be used in hospital and research laboratory.

Principle of Viable CD34+ Cell Analysis

The ADAMII™-CD34 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.

Frame Image (Bright/ FITC/ PE)



A) Bright - Total cells are counted and the cells and debris are iscriminated 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).

Introduction

Background Accurate determination of CD34+ cell numbers is of considerable clinical importance and essential in the field of stem cell transplantation. Several flow cytometry assays for enumerating CD34+ cell have been proposed, but differences in gating methods between users lead to variability in the results. Also flow cytometry assays require expensive instrumentation, have high reagent costs, and, in most cases, are technically difficult. Therefore, a new instrument that produces CD34+ cell counts more simply and reproducibly is required.
Aim We previously developed a new CD34+ cell counting device (ADAMII™-CD34) that uses a microchip and microscopic cell counter. Our aim here was to evaluate the ADAMII™-CD34 as a CD34+ cell counter by comparing it with a conventional flow cytometry.

Method

Samples

- Peripheral blood stem cell (PBSC) samples from 17 patients with hematological malignancies and 11 Cryopreserved umbilical Cord Blood (UCB) samples.
- 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.

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.

Flow cytometry

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

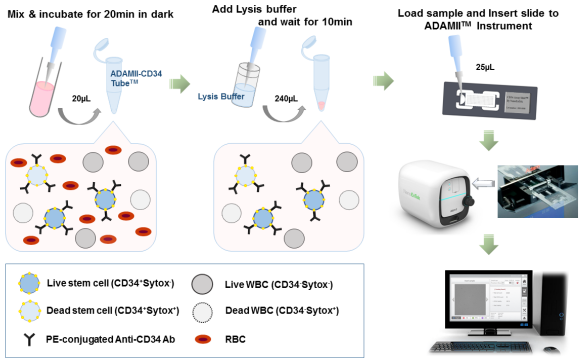


Figure 1. Description of ADAMII™-CD34 Counting Assay

Results

Method Comparison

Linear regression analysis revealed a close correlation between the data for CD34+ cell fractions (%) obtained with the ADAMII™-CD34 and those obtained with the FACSCalibur™ for both PBSCs (r2 = 0.98) and UCBs (r2 = 0.93). The two methods also gave very similar results for the viability of the PBSCs (r2 = 0.94).

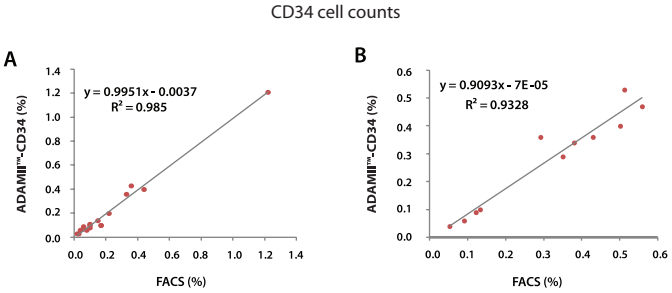


Figure 2. The correlation of CD34 cell counts between the ADAMII™-CD34 and the FACSCalibur™. A) Fresh PBSC, B) Frozen cord blood

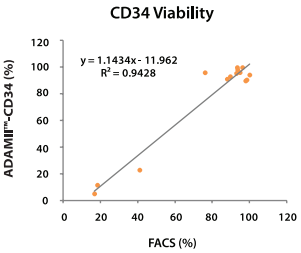


Figure 3. The correlation of CD34 Viability between the ADAMII™-CD34 and the FACSCalibur™

Dilution Linearity

· To assess linearity, samples were serially diluted with mononuclear cells separated from the peripheral blood of a healthy adult.
· A dilution test of the ADAMII™-CD34 method gave a linearity coefficient of r2 = 0.99.

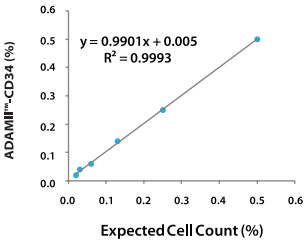


Figure 4. The correlation between CD34 counts as determined by the ADAMII™-CD34 and the expected CD34 counts in diluted samples.

Reproducibility

· To assess reproducibility, some samples were counted 10 times, and coefficients of variations (CVs) were calculated.
· Four PBSC dilutions at CD34+ cell concentrations of 0.44, 0.18, 0.08 and 0.03% had CVs of 7.86, 13.66, 20.83 and 38.46%, respectively.

Table 1. Reproducibility of the same sample tested 10 times

FACS	ADAMII™- CD34 (Repeated 10 times)		
Mean (%)	Mean (%)	SD	CV
0.03	0.03	0.0001	38.46
0.08	0.09	0.0002	20.83
0.18	0.15	0.0002	13.66
0.44	0.41	0.0003	7.86

Summary / Conclusion

We have established a close correlation between CD34+ cell counts and viability assays with the FACSCalibur™ and the ADAMII™-CD34. This suggests that the ADAMII™-CD34+ cell counting device could be useful for stem cell assays given its advantages of reproducibility, accuracy, convenience, and low expensive.