A NOVEL METHOD TO IDENTIFY STEM CELLS IN CORD BLOOD USING THE IMAGESTREAM® 100

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ABSTRACT

Flow cytometry is a standard tool in cell identification and quantification by immunofluorescent staining. However, conventional flow cytometry does not allow direct morphological characterization of cells. The ImageStream® 100 (Amnis Corp.) was developed for simultaneous high-speed multispectral imaging of cells in brightfield, darkfield and fluorescence channels. The multimode imagery allows both photometric and morphometric methods to discriminate cell types and features in heterogeneous populations of cells. The aim of the study was to compare the percentage of CD34 positive stem cells in cord blood using conventional flow cytometry and the ImageStream® 100. Seven discarded cord blood samples of 300 uls were obtained from the New Jersey Cord Blood Bank. The cord blood was lysed with ACK buffer and stained with hematopoietic markers CD45, CD34 (stem cells) and viability stain 7-AAD. The samples were analyzed by an independent lab using the ISHAGE protocol and at Coriell using the ImageStream® 100. Stem cells were determined at Coriell by CD34 positive staining, low granularity and cell morphology parameters using the IDEAS™ analysis software. There were no significant differences in cell viability or CD34 count using the two methods. By flow cytometry 0.40 ± 0.28% of the cells were CD34 positive compared to 0.28 ± 0.1% using the ImageStream® 100. This platform gives the ability for the first time to view these cells both as a population and as individual cells, and apparent CD34/CD45 double positive cells were revealed as cell doublets. Thus, the Imagestream® 100 is a useful new tool for cell characterization.

INTRODUCTION

Hematopoietic stem cells (HSC) are found in human umbilical cord blood and can be an alternative to bone marrow for HSC transplantation. The quality of the cord blood unit for clinical transplantation is assessed by the number of HSC. CD34 antigen is expressed on HSC which is restricted to early progenitors of all lineages. The CD34+ cell population is a rare event, which represents less than 1% of the total leukocytes in the cord blood. This has presented a challenge to the development of standardized, robust protocols for the quantitation of HSC. A standardization of the CD34 assay has been implemented with the dual-platform ISHAGE (International Society for Hematotherapy and Graft Engineering) gating strategy. Additional problems can occur with cord blood due to the presence of variable numbers of nucleated red blood cells, increased apoptosis, cell death and debris, which can potentially confound the analysis. A novel method to analyze $CD34^+$ cells could be performed using the Imagestream system, which combines the capabilities of microscopy and flow cytometry to produce high resolution brightfield, darkfield and multiple fluorescence images of cells. Our goal in this study was to assess the capabilities of the Imagestream® 100 (IS 100) to determine the percentage of $CD34^+$ cells in cord blood and to compare this with the ISHAGE flow cytometry method.

MATERIALS AND METHODS

Cord Blood Specimen Umbilical cord blood samples (n=7) were collected from full-term deliveries. Three cord blood samples were processed fresh and four were frozen and recovered. All procedures used for collecting and processing cord blood were based on the standard operating procedures of the New Jersey Cord Blood Bank. Aliquots of the cord blood from each donor were lysed with ACK lysing buffer.

CD34 staining and Imagestream The samples were stained with anti-CD34-PE (HSC) or 7-AAD (viability stain) and fixed with 2% paraformaldehyde. Fixed cells at 2 x 10^7 cells per ml were run at 100 cells per second capturing 40,000 events on the Imagestream® 100 and then analyzed using the the Imagestream Data Analysis and Exploration Software (IDEAS). Single cells were identified and these events were used to identify CD34+ and low side scatter cells. CD34+ cell morphology was examined to eliminate false positives. A subset of the samples were also stained with anti-CD45-FITC (leukocytes). CD34+ cells were identified with having dim CD45 expression, low side scatter and high CD34 expression, which is similar to the ISHAGE strategy.

ISHAGE flow cytometry protocol The samples were independently analyzed for the percentage for $CD34^+$ cells using the ISHAGE sequential gating strategy. This procedure stains the white blood cells with anti-CD45-FITC and anti-CD34-PE. Samples were analyzed by flow cytometry to identify cells that have dim CD45 expression and low side scatter. These events were displayed on a CD34 vs SSC dot plot to allow gating of the cluster of $CD34^+$ events.

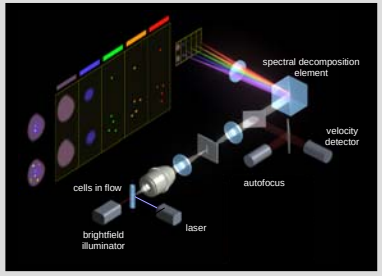


Fig. 1. Schematic representation of the Imagestream® 100

RESULTS

- CD34 staining analyzed using the Imagestream® 100 gave estimates of 0.31 ± 0.15% cells being $CD34^+$ (Table 1 and Fig. 2), which was similar to the ISHAGE flow protocol of 0.40 ± 0.28%.
- Dual CD34 and CD45 staining analyzed using the Imagestream® 100 produced a similar percentage of $CD34^+$ cells (0.28 ± 0.1%) (Fig. 3) compared to the ISHAGE protocol and the single staining Imagestream® 100 protocol (Table 1).
- There was reduced total nucleated cell viability (TNC %V) in recovered frozen cord blood (90.62%) compared to fresh (99.41%). Viability using the IS 100 was comparable to results determined by the ISHAGE protocol (Table 1).
- The Imagestream® 100 allowed the capability to: 1) identify single or doublet cells, cell debris and ghost cells in cord blood ; 2) analyze cell images of CD34 stained cells and discriminate $CD34^+$ staining from contaminated events such as platelets, platelet aggregates and other debris that binds to the CD34 antibody (Fig 2D and 3F); 3) examine cell morphology of double stained cells and to discriminate whether there is co-localized staining (eg $CD34^+$, $CD45^+$) on the cells or the occurrence of doublets that are singularly stained (Fig. 3G).

Table 1 Comparison of CD34 identification by flow or Imagestream® 100 (* recovered frozen cord blood)

DONOR NUMBER	$CD34^+$, $CD45^{low}$ (Flow) (TNC % V)	$CD34^+$, SSC^{low} (IS 100)(TNC % V)	$CD34^+$,$CD45^{low}$, SSC^{low} (IS 100)
459*	0.39 (81.1)	0.31 (87.6)	0.32
744*	0.19 (95.9)	0.23 (89.3)	0.24
260*	0.20 (91.6)	0.14 (95.3)	0.10
012*	0.98 (95.7)	0.51 (88.1)	0.36
151	0.51 (99.6)	0.45 (nd)	0.39
250	0.17 (99.7)	0.14 (nd)	0.19
130	0.37 (99.9)	0.37 (nd)	0.34

Fig. 2 Identification of $CD34^+$ cells in cord blood (163) using the IDEAS software by gating for single cells (A), low SSC and CD34 intensity (B). C shows a discrete population of $CD34^+$ cells in the cord blood. Images captured by the Imagestream® 100 of $CD34^+$ cells (D).

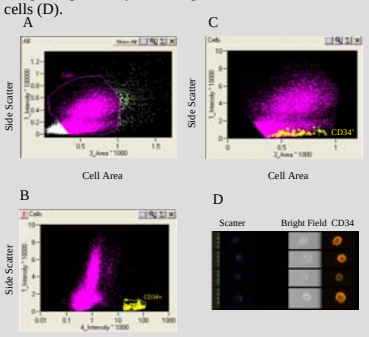
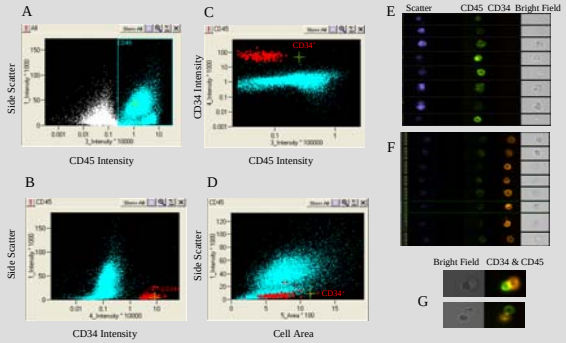


Fig. 3 Identification of $CD34^+$ cells in cord blood (150) using the IDEAS software by gating for CD45 expression (A), low SSC and CD34 intensity (B). C shows a discrete population of $CD34^+$, $CD45^{low}$ and with SSC^{low} observed in the $CD45^+$ population of cells (D). Images captured by the Imagestream® 100 of $CD45^+$ cells (E), $CD34^+$, $CD45^{low}$ cells (F) and $CD34^+$,$CD45^+$ doublets (G).



CONCLUSIONS

This study demonstrated the ability of the Imagestream® 100 to identify and quantitate the percentage of $CD34^+$ stem cells in cord blood with comparable results to flow cytometry methods. In contrast to flow cytometry, Imagestream fluorescence data is linked to cell imagery and the morphology of gated populations like CD34+ cells can be viewed directly. The Imagestream® 100 could be a useful tool in identifying rare events such as stem cells in a heterogeneous populations of cells.