

# Evaluation of an Automated Instrument for Viability and Concentration Measurements of Cryopreserved Hematopoietic Cells

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## ABSTRACT

Two important parameters for determination of deleterious effects of cellular processing on hematopoietic progenitor cells are cell viability and concentration. The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins Hospital evaluated the Beckman Coulter Vi-Cell automated instrument for the measurement of these two parameters. Using 33 thawed hematopoietic progenitor cell samples, automated Vi-Cell viability results were compared to those obtained using the standard trypan blue manual method. In addition, cell concentrations from these samples were compared with results from the Model Z2 Coulter Counter. Chinese Hamster Ovary cells were used for the evaluation of Vi-Cell linearity at the Beckman Coulter Cellular Analysis Development Center. Significant correlation was obtained when the two methods were compared for both cell concentration and percentage viability ( $P < .0001$ ). The results of the linearity study indicated that the Vi-Cell is linear from approximately  $5 \times 10^4$  to greater than  $1 \times 10^7$  cells/mL. The Vi-Cell uses sample volumes as low as 0.5 mL; cell diameters may be 2 to 70 microns. The Vi-Cell automated instrument offers many significant advantages for cell analyses in today's busy laboratory environment. *Lab Hematol.* 2004;10:109-111.

**KEY WORDS:** Cell viability · Hemacytometer · Vi-Cell

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## INTRODUCTION

The health of mammalian cells in culture is often assessed by the determination of cell concentration and percentage of viable cells. Also, cryopreserved cells are routinely assayed for percentage viability and concentration, both prior to freezing and after the recovery process [1]. In order to measure the possible deleterious effects of the cryoprotectant on hematopoietic cells removed from storage in liquid nitrogen, the Graft Engineering Laboratory at Johns Hopkins Hospital uses the standard, manual trypan blue vital dye exclusion cell viability assay. Viable cells, due to their intact cell membranes, exclude uptake of the trypan blue stain; nonviable, membrane-porous cells stain blue. This method requires a technologist, using a hemacytometer and a light microscope, to enumerate both stained and unstained cells. The percentage viability is then calculated. Unfortunately, this technique has several major disadvantages. The hemacytometer has a significant accuracy error, due to its subjective nature [2]. In addition, the manual method is tedious and time-consuming for today's busy cell culture laboratory. The Beckman Coulter Vi-Cell (Figure 1) automates the manual trypan blue dye exclusion technique; this instrument, in addition to percentage viability, reports both total and viable cell concentrations, as well as other significant parameters such as cell size and circularity measurements.

The Beckman Coulter Vi-Cell automatically mixes the cell sample with an equal volume of 0.4% trypan blue stain. Cells are drawn through a flow cell of known volume and imaged using a high-resolution ( $1392 \times 1040$  array) CCD camera. Pixels are the smallest elements of the image. Grayscale values define the brightness of each pixel. The



FIGURE 1. The Beckman Coulter Vi-Cell.

grayscale goes from 0 (black) to 255 (white). The stained, nonviable cells appear darker and thus have lower grayscale values. Those cells with higher grayscale values are considered viable. The Vi-Cell camera image screen shows real-time images of the cell population (Figure 2). Up to 100 images may be viewed and archived for future reanalyses.

The objective of this research was to compare Vi-Cell percentage viability results of a variety of recovered hematopoietic cell samples, cryopreserved and stored in liquid nitrogen, to data obtained using the manual method. In addition, cell concentrations of these samples were compared with the results from the Beckman Coulter Model Z2.

## METHODS

Thirty-three cryopreserved hematopoietic progenitor cell samples were thawed and tested for viability using the manual method and the Beckman Coulter Vi-Cell. Cell concentrations were analyzed using the Vi-Cell and the model Z2 Coulter Counter. Cell types included: (1) bone marrow cells ( $n = 16$ ), (2) mononuclear cells from multiple myeloma and acute myelocytic leukemia patients ( $n = 7$ ), (3) peripheral blood progenitor cells ( $n = 9$ ), and (4) mononuclear cells from peripheral blood of an acute lymphocytic leukemia patient ( $n = 1$ ). Light density mononuclear cells were isolated using the standard Ficoll-Hypaque (Pharmacia) density gra-

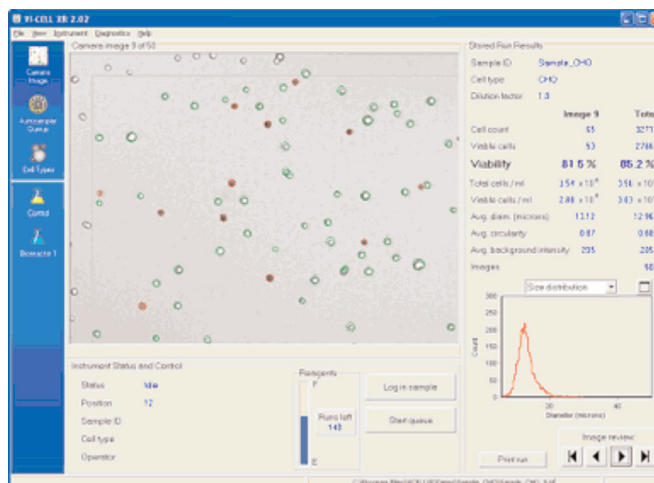


FIGURE 2. Vi-Cell Camera Image Screen.

dient method [3]. Peripheral blood progenitor cells were collected by apheresis;  $CD34^+$  cells were separated using the Baxter magnetic cell separation system [4].

When more than trace amounts of red blood cells were present, they were lysed using ammonium chloride. The samples were washed in phosphate buffered saline and resuspended in Isoton II. Initial cell concentrations were measured using the Beckman Coulter model Z2. Fifty microliters of sample was mixed with 50  $\mu$ L of trypan blue and loaded into a standard hemacytometer. For the manual hemacytometer method, approximately 100 cells were counted per sample. One milliliter of each cell sample was placed into a sample cup and placed onto the Vi-Cell sample carousel for automated analyses. All samples were assayed within 30 minutes of thawing and within 3 minutes of the addition of trypan blue.

At the Beckman Coulter Cellular Analysis Center in Miami, FL, USA, Chinese hamster ovary (CHO) cells grown in standard tissue culture media were used to test the linearity of the Vi-Cell. Dilutions in phosphate buffered saline were prepared at 1:2, 1:5, 1:10, 1:20, 1:50, 1:100, and 1:200. All samples were analyzed on the Vi-Cell.

## RESULTS

There was excellent correlation of hematopoietic cell concentration results between the Vi-Cell and the Z2 Coulter Counter (Table 1).

Significant agreement of percent viability data also was observed when comparing the Vi-Cell to the manual, hemacytometer method (Table 2).

For viability, a mean of 2888 cells were counted per sample (range 61-11,535) using the Vi-Cell. Approximately 100 cells were analyzed with the manual method. The concentra-

TABLE 1. Concentration Comparison\*

Instrument	N	Mean, cells/mL	SD
Vi-Cell	33	$1.2 \times 10^7$	$2.1 \times 10^7$
Z2	33	$1.1 \times 10^7$	$2.1 \times 10^7$

\*P < .0001.

tion linearity is shown in Figure 3. Using CHO cells, the Vi-Cell demonstrated linearity from approximately  $5 \times 10^4$  cells/mL to greater than  $1 \times 10^7$  cells/mL.

DISCUSSION

The results of this study indicate that the Beckman Coulter Vi-Cell has many features that are attractive to the busy cell-processing laboratory. As mentioned, there is significant variation of hemacytometer viability results among different laboratory personnel when analyzing the same cell sample. It is a highly subjective assay. The Vi-Cell removes the subjectivity inherent in the method by providing an objective analysis of cellular viability. The manual method is time-consuming. The Vi-Cell provides data and cleans the flowcell in less than 3 minutes. In addition, the Vi-Cell tests 15 to 30 times the sample volume of a hemacytometer. The larger number of cells included in the analysis provides much greater statistical confidence than results from the manual method.

Cell concentration may be obtained by direct hemacytometry; however, in 1988, the International Council For Standardization In Hematology (ICSH), regarding a reference method for red and white blood cell enumeration, recommended that “chamber counting, due to imprecision and inaccuracy, be replaced by a single channel, semi-automated electronic counter using the aperture-impedance principle” [5]. The Model Z2 meets the ICSH instrument specifications and also conforms to the ASTM F2149-01 Standard Test Method for Automated Analyses of Cells [6].

TABLE 2. Viability Comparison\*

Instrument	N	Mean, %	SD
Vi-Cell	33	47.50	21.8
Hemacytometer	33	46.50	20.4

\*P < .0001.

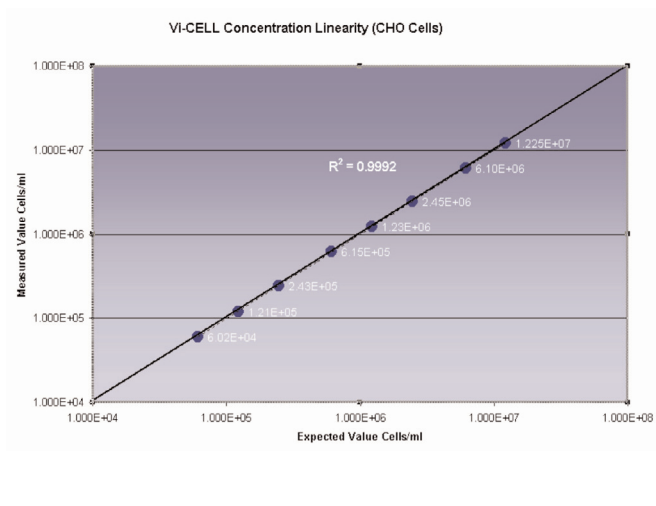


FIGURE 3. Vi-Cell concentration linearity (Chinese hamster ovary cells).

The Vi-Cell demonstrated significant correlation with a wide range of cell concentrations concurrently analyzed on the Z2 instrument.

In addition to results correlation with other methods, the Vi-Cell instrument offers today’s cell processing laboratories the advantages of “walk away” sample analyses, real-time cellular imaging, and data archiving for future reanalyses. The instrument uses as little as 0.5 mL of sample volume. Linearity is  $5 \times 10^4$  to greater than  $1 \times 10^7$  cells/mL, and cell diameters may range from 2 to 70 microns.

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