

APPLICATION INFORMATION

Cell Viability

AUTOMATED CORD BLOOD CELL VIABILITY AND CONCENTRATION MEASUREMENTS USING THE VI-CELL™ XR

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Introduction

Stem cells, due to their differentiation into mature blood cells, are the key to successful bone marrow transplantations. More recently, it has been found that umbilical cord blood is also a plentiful and rich source of hematopoietic stem cells.⁽¹⁾ Thus, both bone marrow and cord blood are used in the treatment of numerous cancers, immunological disorders, and certain genetic diseases.

Cord blood stem cells, however, offer important advantages over cells isolated from bone marrow. Stem cells from cord blood are much easier to secure since they are readily obtained from the umbilical cord and placenta at the time of delivery, whereas harvesting stem cells from bone marrow requires a surgical procedure (usually under general anesthesia). For a bone marrow transplant to succeed, there must be a nearly perfect match of tissue proteins—called antigens—between the donor and recipient. Cord blood stem cells “take” even when there are partial mismatches.⁽²⁾ Hence, more recipients benefit from stem cells infused from cord blood.

Currently, many facilities provide parents the option to store, or bank, their newborn baby’s cord blood.⁽³⁾ When cells are banked—usually in liquid nitrogen—two parameters must be accurately assayed. These measurements are cell concentration and percentage of cell viability.⁽⁴⁾ These measurements are performed prior to storage and after the thawing process. Cells may decrease in both number and viability, mainly due to the cryopreservative employed in the freezing process (usually DMSO).

The Vi-CELL™ XR (Figure 1) from Beckman Coulter automates the manual trypan blue vital dye exclusion method for cell viability determinations. In addition, the instrument provides an objective measurement of cellular concentration. As mentioned, these are two critical parameters required in the cord blood cell banking process.

The objective of this work was to describe a method for cord blood sample preparation, and develop a cell type, accurate set of instrument parameters, for the Vi-CELL XR.



Figure 1. The Vi-CELL XR.

Materials and Methods

A sample of cord blood was obtained from Baptist Hospital, Miami, FL, by the SAS laboratory at Beckman Coulter. The blood was diluted 1:1 using room-temperature phosphate buffered saline. The standard Ficoll[®] gradient separation method was used to isolate the mononuclear cells.⁽⁵⁾ Isolated cells were washed once in PBS and resuspended in 2 mL of Isoflow[®] (Isoton[®] II). A 1:10 dilution of the cell suspension was prepared by placing 100 μ L of cells into 900 μ L of Isoflow in a standard Vi-CELL[™] sample cup. The Vi-CELL XR was used to develop the cord blood cell type. Prior to sample analysis, the Vi-CELL[™] concentration control was assayed.

Results

Figure 2 shows the results of the Vi-CELL concentration control. Figure 3 illustrates a cord blood cell image on the Vi-CELL XR. Figure 4 shows the cell type parameters used to analyze cord blood cells. The size range was set from 3 to 25 microns. Cell brightness: 85; cell sharpness: 100; viable cell spot brightness: 65; viable cell spot area: 5.

Conclusions

Cord blood stem cell infusions have significant advantages over bone marrow transplants. Viability and concentration of cord blood cells may be assayed using the Vi-CELL XR from Beckman Coulter. The cell type settings for cord blood samples were determined. Size range 3 to 25 microns. Imaging parameters: Cell brightness: 85; cell sharpness: 100; viable cell spot brightness: 65; viable cell spot area: 5.

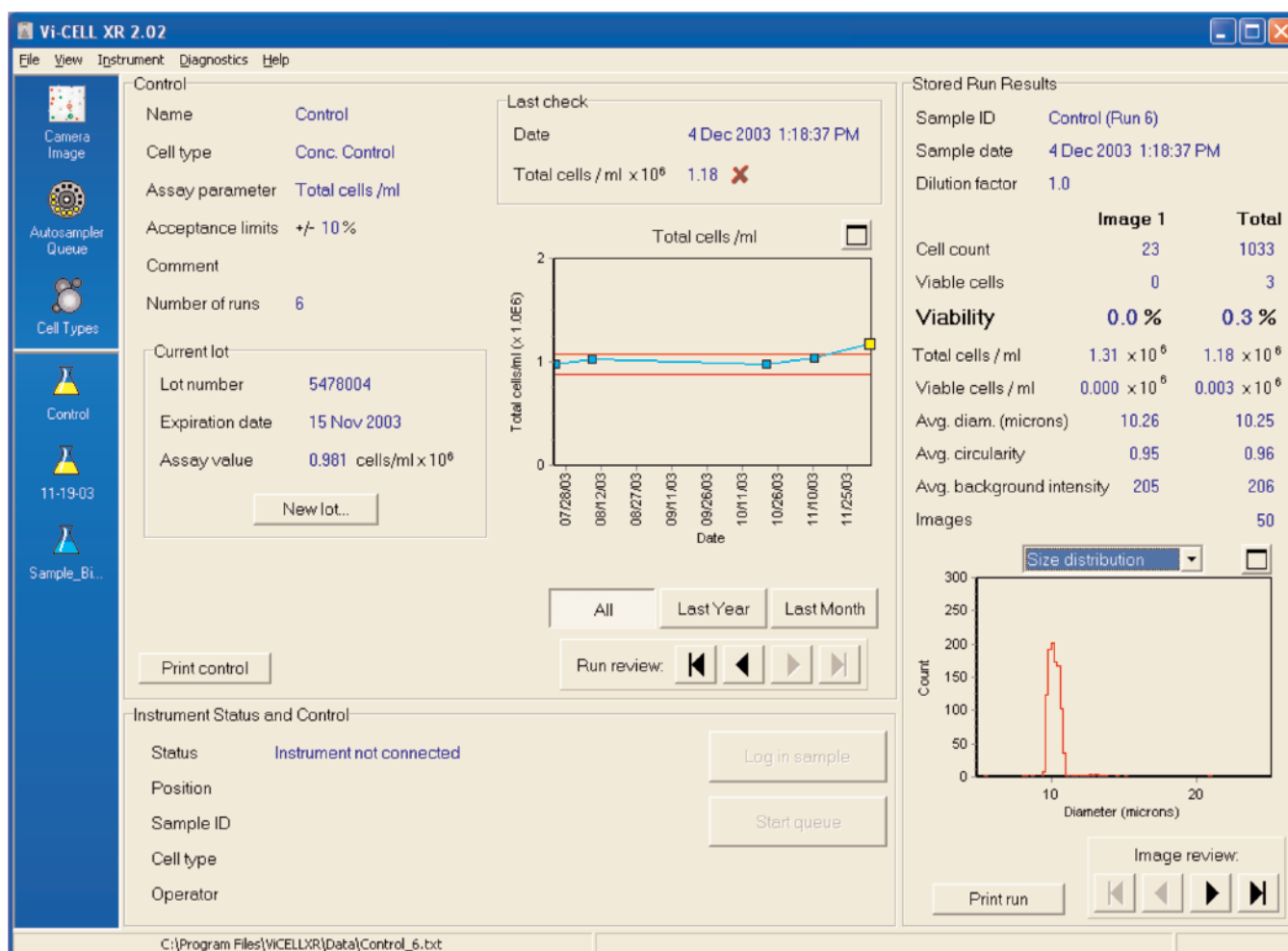


Figure 2. Results of the Vi-CELL concentration control.

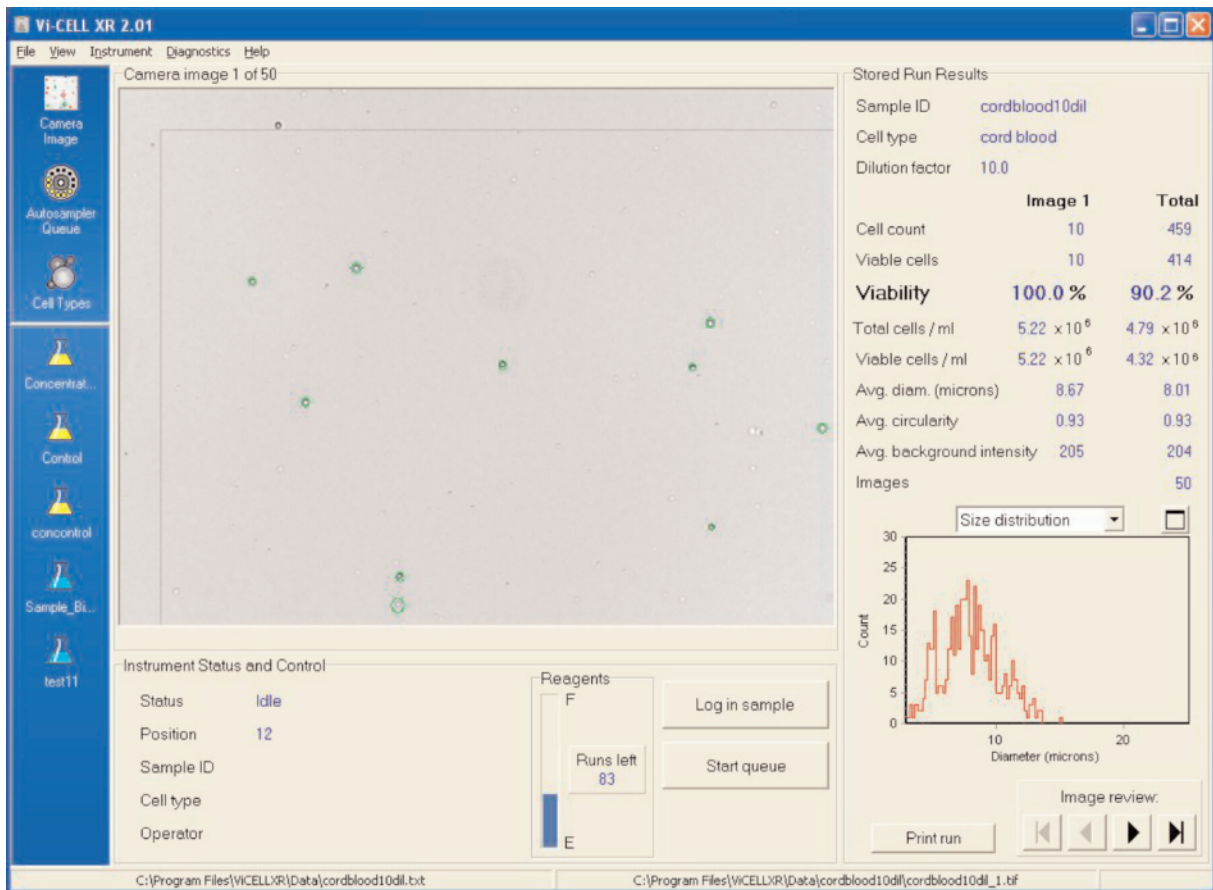


Figure 3. A cord blood cell image on the Vi-CELL™ XR.

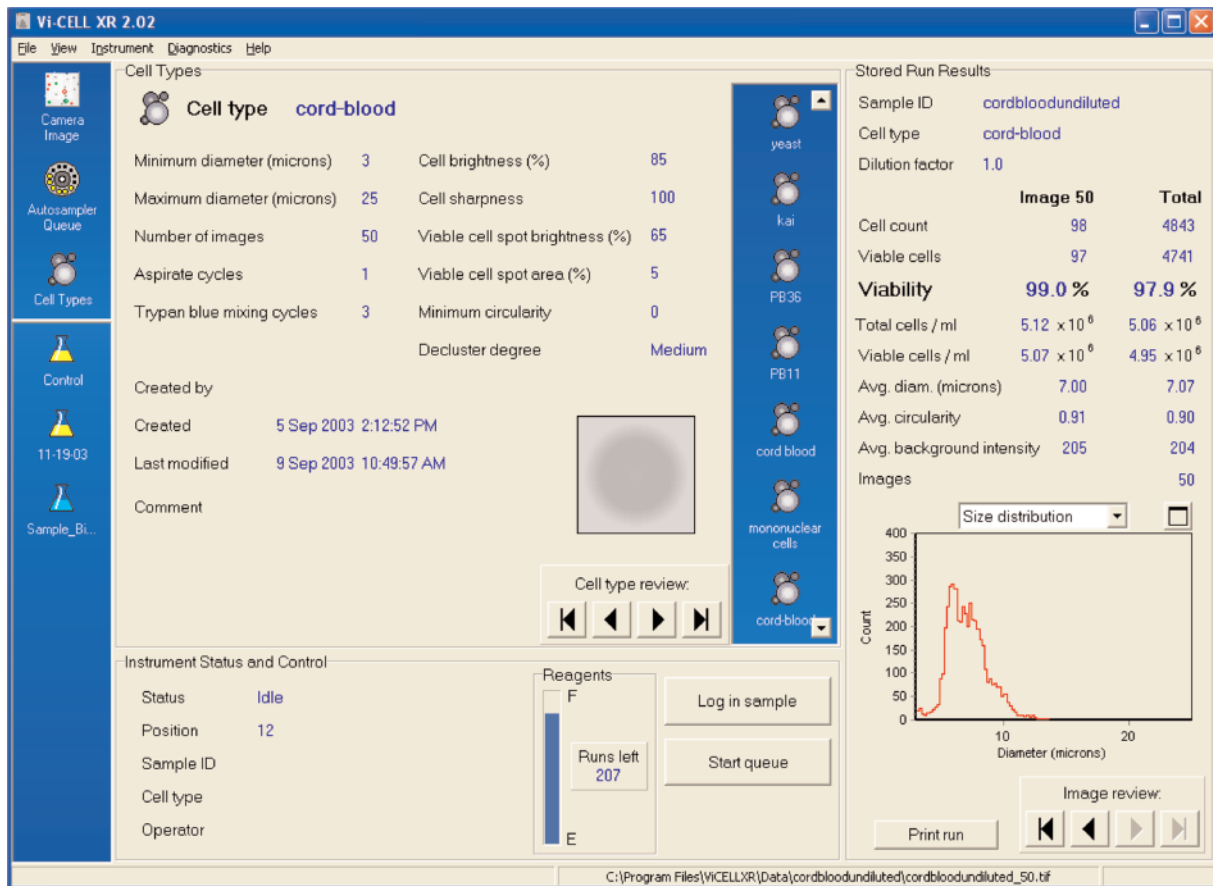


Figure 4. Cell type parameters used to analyze cord blood cells.

References

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