APPLICATION INFORMATION

Cell Viability

STEM CELL ANALYSES USING THE VI-CELL™ XR

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Introduction

Stem cells, which resemble white blood cells with very small nuclei and cytoplasm, have the capacity to differentiate into any of the mature blood cells. Very primitive, pleuripotent stem cells have the capability of differentiation into other cell types, such as heart and nerve, as well.[1]

Since 1968, stem cells in bone marrow have been used in treatment of leukemias, lymphomas, and immune deficiency disorders.[2] More recently, cord blood and peripheral blood stem cells have been utilized. Currently, the main sources for stem cells are bone marrow, cord blood, and peripheral blood.

Chemotherapy and radiation used in cancer therapy have an adverse effect on bone marrow. Stem cells have been used to repopulate a patient's bone marrow subsequent to high-dose chemotherapy or radiation treatments. The choice of the source of stem cells is often based on such factors as the health of the bone marrow cells or the difficulty of stem cell collection. For example, harvesting stem cells from the bone marrow may require general anesthesia. Peripheral blood stem cells are collected, over several days, by the apheresis process. Also, if the patient's bone marrow contains cancerous cells, sometimes healthy cells may be obtained from peripheral blood.

Often the stem cells, regardless of source, are frozen for future cellular transplantation. Upon thawing, these cells must be assayed for both percentage viability and concentration.[3]

Many facilities employ the manual, trypan blue method for these measurements. The Beckman Coulter Vi-CELL™ XR (Figure 1) automates the manual trypan blue vital dye cell viability method. The instrument removes the subjective variation among operators inherent in manual cell enumeration using a microscope. This Application Bulletin demonstrates that stem cell percentage viability and concentration, from the above-mentioned sources, may be measured accurately and precisely using the Beckman Coulter Vi-CELL XR.

Figure 1. *The Vi-CELL XR.*

Methods

Twenty-five cryopreserved samples of bone marrow (n=16) and peripheral blood stem cells (n=9) were tested at Johns Hopkins Hospital in Baltimore, MD. The standard Ficoll gradient separation method was employed for cell isolation.[4] Samples containing

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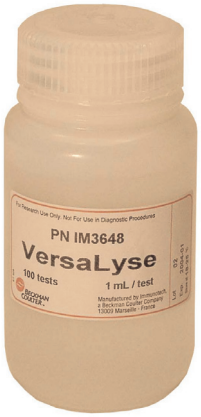

Figure 2. *Beckman Coulter Versalyse.*

more than trace amounts of red blood cells were lysed using ammonium chloride. Beckman Coulter manufactures an ammonium chloride red blood cell lysing reagent, IO Test 3, PN IM3514. Alternatively, Beckman Coulter VersaLyse (Figure 2), PN IM3648, may be used. Both of these reagents cause no damage to the stem cell membranes while efficiently removing the red blood cells from the analyses.

The cord blood sample, obtained from Baptist Hospital, Miami, FL, was analyzed in our laboratory at the Beckman Coulter facility in Miami.* Also, a sample of bone marrow, from the Diabetes Research Institute in Miami, was tested using a Vi-CELL™ XR. Cells were isolated using the Ficoll gradient.

The Vi-CELL XR sample counts at Johns Hopkins were compared with the results of the Z2™ COULTER COUNTER® (Figure 3). Vi-CELL XR viability data was compared to the manual, trypan blue method.

Results and Discussion

Significant correlation, $p < 0.0001$, was obtained when comparing the two methods, the Z2 and V-CELL, for cell count.

There was also significant correlation, $p < 0.0001$, observed between percentage viability from the Vi-CELL XR and the manual technique.

Figure 4 shows the Vi-CELL XR (autosampler configuration) image screen with peripheral blood stem cells isolated at the Johns Hopkins Hospital laboratory. The size distribution graph shows this cell population to be 5 microns and greater.

For the cells analyzed at Johns Hopkins, the mean number of cells per sample was 2888.

Figure 3. *Z2 COULTER COUNTER.*

Approximately 100 cells were counted using the hemacytometer. The higher number of cells tested on the Vi-CELL XR results in data of higher statistical confidence than that of the manual method.

Figure 5 shows the Vi-CELL XR camera image screen with cells isolated from a sample of bone marrow. Note how "clean" the cell preparation appears. There are few, if any, residual red blood cells or other contaminating cells present. The lower size of this cell preparation is about 3 microns.

As mentioned, cord blood provides a rich source of stem cells. Some feel that cord blood cells are more "immunologically naïve," thus they do not provoke as strong a negative response when transplanted as do cells from other sources. Figure 6 illustrates cord blood cells analyzed on a Vi-CELL XR.

Conclusions

The Beckman Coulter Vi-CELL automates stem cell viability and concentration measurements from bone marrow, peripheral blood, and cord blood. It reports objective, accurate results, removing the subjective nature inherent in the manual method.

Due to the lower size—approximately 3 microns—of most stem cell populations, it is recommended that the Vi-CELL XR model be used. The concentration results (n=25) from the Vi-CELL XR and the model Z2 COULTER COUNTER showed excellent correlation. There was significant correlation between the percentage viability results (n=25) from the Vi-CELL XR and the manual hemacytometer method.

* For a detailed protocol on cord blood Vi-CELL analyses, see the Beckman Coulter *Application Information Bulletin A-1979A,* Automated Cord Blood Cell Viability and Concentration Measurements Using the Beckman Coulter Vi-CELL XR.

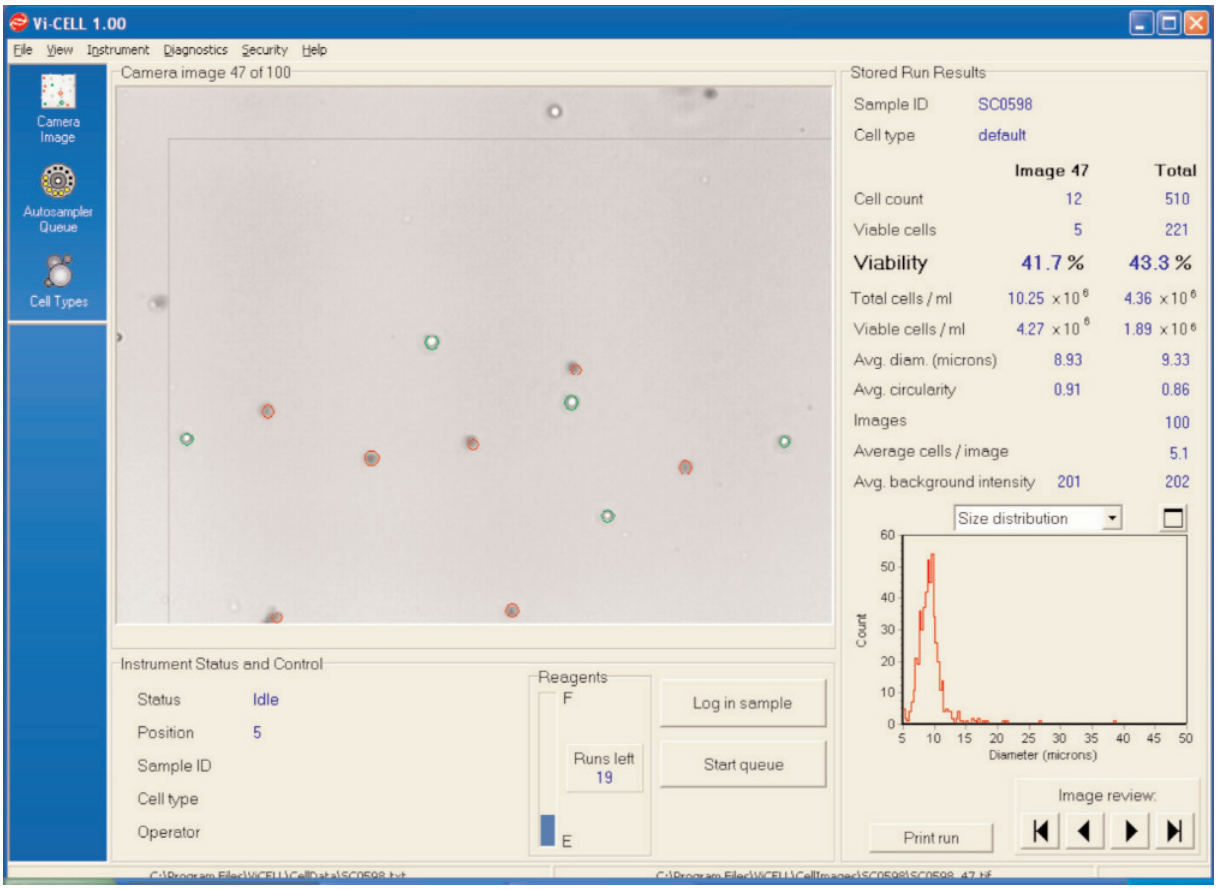

Figure 4. *Peripheral blood stem cells isolated at Johns Hopkins Hospital laboratory.*

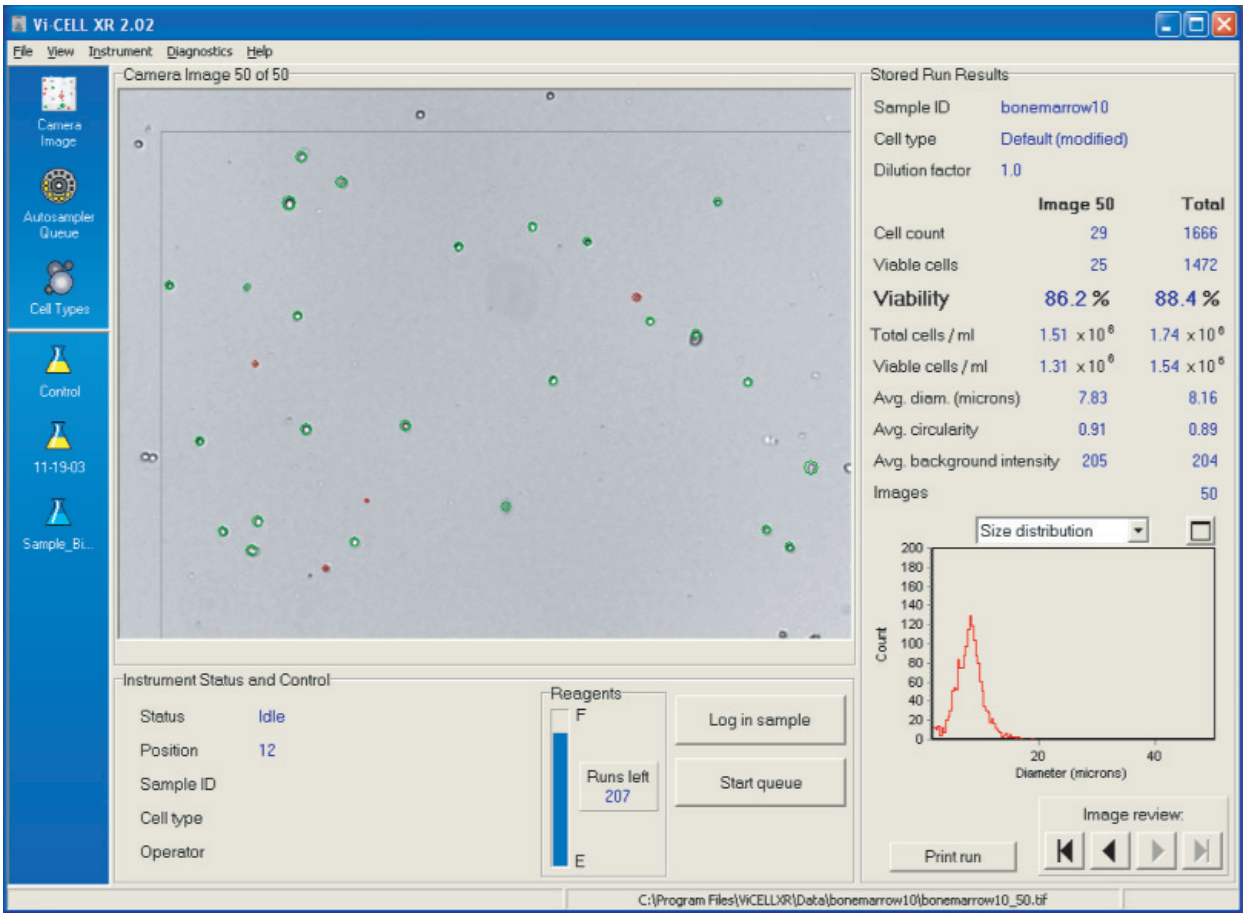

Figure 5. *Cell isolated from a sample of bone marrow.*

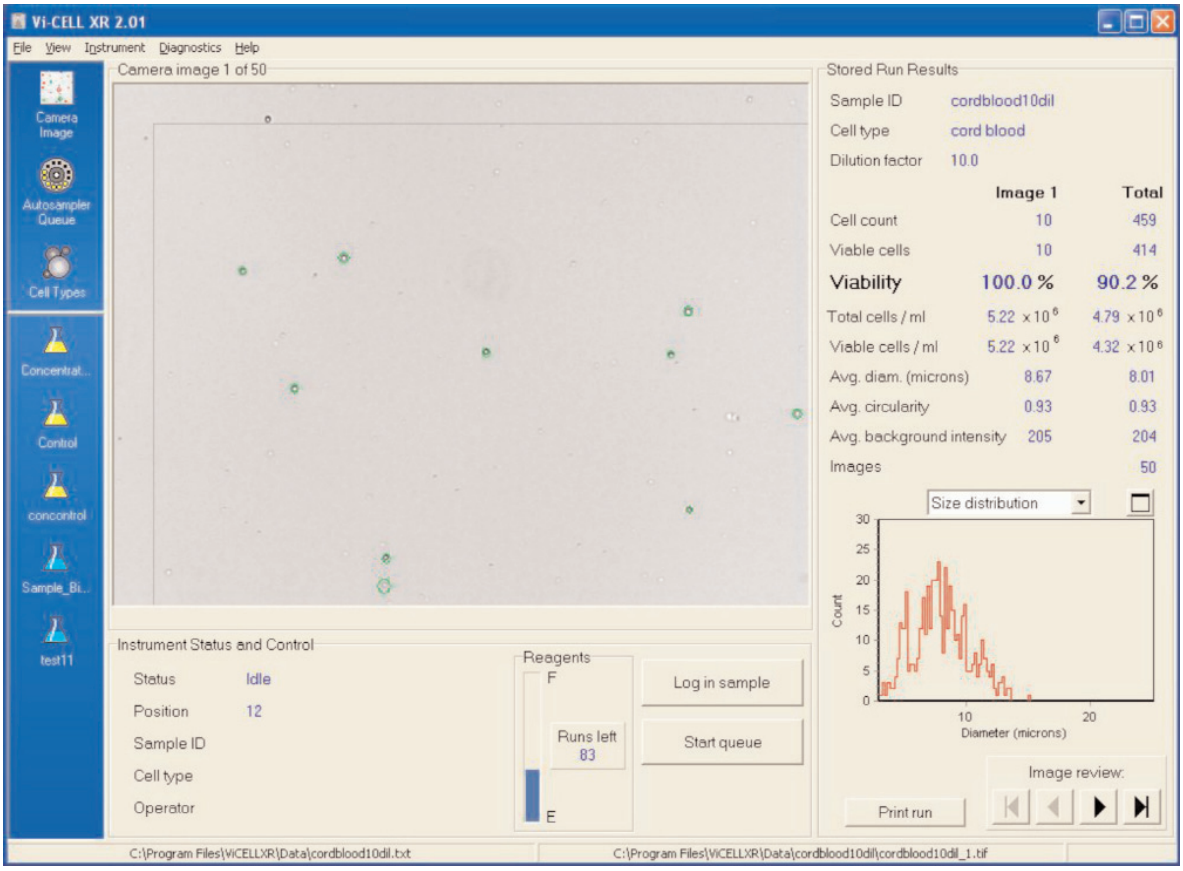

Figure 6. *Cord blood cells analyzed on the Vi-CELL™ XR.*

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