APPLICATION INFORMATION

Cell Viability

USE OF BECKMAN COULTER REAGENTS FOR AUTOMATED, WHOLE BLOOD, WHITE BLOOD CELL VIABILITY MEASUREMENTS

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Introduction

The Beckman Coulter Vi-CELL™ Cell Viability Analyzer (Fig.1) automates the standard, manual trypan blue vital dye exclusion method. The principle of this assay is the vital stain uptake by porous nonviable cells. The Vi-CELL has been extensively used to measure percent viability of cells, such as CHO, employed in recombinant protein production.[1]

Many laboratories still use the manual, hemacytometer method for both concentration and percentage viability measurements on patient blood samples.[2] The Vi-CELL automates this protocol, thus removing the subjective nature of the manual white blood cell (WBC) counting procedure. Hence, a consistent, accurate and reproducible measurement is obtained using the Vi-CELL.

When analyzing a blood sample for WBC viability, the red blood cells (RBC) must be lysed, leaving the WBC population. Unfortunately, many red blood cell lytic agents result in WBC membrane porosity in actively metabolizing, reproducing cells, thus resulting in grossly inaccurate cellular viability data.

Figure 1. *Beckman Coulter Vi-CELL*

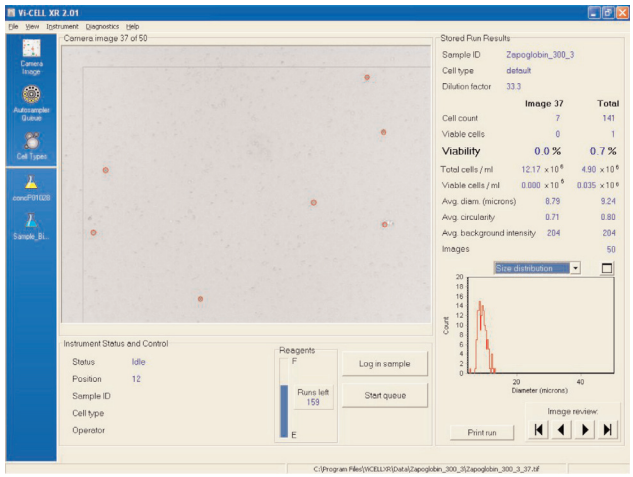

Figure 2. *Zap-OGLOBIN® II*

Methods and Discussion

When analyzing WBCs in whole blood, selection of an appropriate lysing solution is critical to result accuracy. The lysing mechanism must not result in damage to the WBC membrane; as mentioned, porosity of the membrane due to lyse results in the uptake of the vital dye, trypan blue, by actively metabolizing, viable cells. Damaged WBCs will appear as non-viable. Beckman Coulter Zap-OGLOBIN® II Lytic Reagent is an excellent solution often used to lyse RBCs. This reagent is an excellent choice for WBC enumeration using the Z™ Series models, but not the Vi-CELL. Fig. 2 illustrates a WBC image on the Vi-CELL using Zap-OGLOBIN II as the RBC lytic agent. The mechanism of this lyse results in the

REFERENCES
1. Szabo, SE. Bioprocessing. Automating the Assessment of Cell Viability. Tutorial: Vi-CELL Instrument Overcomes Limitations of Manual Methods. Genetic Eng. News. 23, No.10, May 15, 2003.
2. Fiorino, S., Bitzan, J., Loper K., M. Zahurek, S. Szabo, R. Jones, I. McNiece. Evaluation Of An Automated Viability Analyzer. Johns Hopkins Med. Schl. Abstr. ISCT., May, 2003.
3. Ibid

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removal of the WBC membranes. Thus, all cells viable and non-viable now stain blue due to the uptake of the trypan blue. The Vi-CELL, using annotation, circles non-viable cells red; viable are circled in green. Beckman Coulter's Immuno-Lyse and OptiLyse® C were also evaluated and found to significantly reduce cell viability via WBC membrane dam-

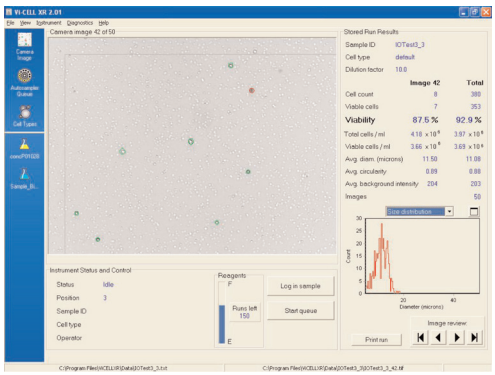

Figure 3. *IO Test® 3*

age occurring during the lyse procedure. Many laboratories employ an ammonium chloride lysing solution.[3] Beckman Coulter manufactures an ammonium chloride-based reagent, IO Test® 3 Lysing Solution, PN IM3514. The reagent is supplied as a 10X concentrated product. A 1X solution should be prepared daily. Figure 3 shows the results of the same blood sample using IO Test 3 as the lysing solution. This lyse leaves the remaining white cell population relatively unperturbed, allowing an accurate WBC viability measurement. A new lysing reagent has been released by Beckman Coulter. This product is VersaLyse, PN IM3648. Figure 4 shows WBCs in whole blood using VersaLyse. Note the "background" of RBC "ghosts" that is produced using this reagent. "Ghosts" may also be seen when using the IO Test 3 solution. These structures are the RBC "re-sealed" membranes after RBC hemoglobin release. The contrasting WBCs, accurately captured by the Vi-CELL's imaging algorithm, are very apparent.

The procedure for sample preparation using VersaLyse requires minimal labor. It is as follows:

- Add 100 microliters whole blood to a tube or Vi-CELL cup
- Add 1 ml VersaLyse
- Vortex 5 seconds
- Incubate for 10 minutes at room temperature
- Load sample(s) onto the Vi-CELL or Vi-CELL XR

Use the default imaging cell type parameters. The same procedure, with addition of 2mls of lyse to 100 microliters of blood, may be used with IO Test 3.

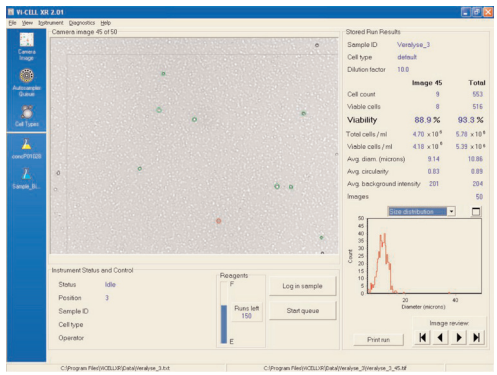

Figure 4. *VersaLyse. Note RBC "ghosts" (white specs)*

Conclusions

The Beckman Coulter Vi-CELL automates the manual WBC viability method.

The selection of the RBC lytic agent is critical to the WBC viability accuracy measurement.

Zap-OGLOBIN II, ImmunoLyse, or OptiLyse C are not suitable for Vi-CELL WBC viability analyses. The Beckman Coulter reagents, IO Test 3 and VersaLyse, were shown to be effective for result accuracy in WBC viability measurements using the Vi-CELL.

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