Accurate Enumeration of CD34+ Cells with the BD™ Stem Cell Enumeration Kit on the BD FACSLyric™ System

Introduction
Hematopoietic stem cells (HSCs) are CD34+ and are responsible for engraftment in the bone marrow transplant setting. Enumerating CD34+ HSCs in peripheral blood, apheresis and cord blood samples provides critical information to the transplant physicians. The number of viable CD34+ cells present in the peripheral blood after mobilization and/or chemotherapy predicts the yield of CD34+ cells in the apheresis product. Additionally, the number of CD34+ cells collected predicts time to engraftment after transplantation. The infusion of a minimum of two million viable CD34+ cells per kilogram patient weight generally enables rapid [10-12 days to 500 neutrophils/µL] and sustained engraftment in the auto-transplant setting. The International Society of Hematotherapy and Graft Engineering (ISHAGE) protocol for CD34+ cell enumeration is the most widely used flow cytometric method in clinical laboratories. BD Biosciences has developed an algorithm for the BD FACSLyric™ system that closely follows the ISHAGE protocol. This study was performed to demonstrate the accuracy of the new assay algorithm to enumerate CD34+ HSCs.

Materials & Samples

<table>
<thead>
<tr>
<th>BD™ Stem Cell Enumeration kit</th>
<th>Samples (50 total):</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD™ Stem Cell Control Kit</td>
<td>15 apheresis</td>
</tr>
<tr>
<td>BD™ Cord &amp; RBC Beads</td>
<td>15 mobilized apheresis</td>
</tr>
<tr>
<td>BD™ FC Beads &amp; 7 Color Kit</td>
<td>15 cord blood</td>
</tr>
<tr>
<td>BD FACSYS™ system</td>
<td>2 bone marrow</td>
</tr>
<tr>
<td>BD FACSuite™ software</td>
<td>3 normal peripheral blood</td>
</tr>
<tr>
<td>BD FACSuite™ software</td>
<td></td>
</tr>
<tr>
<td>BD CellQuest™ Pro software</td>
<td></td>
</tr>
</tbody>
</table>

The samples were stained using the BD™ Stem Cell Enumeration kit. The stained samples were acquired on the BD FACSLyric™ system using BD FACSuite™ software. The cytometer was set up using BD™ C&T RUO beads for daily performance quality control, BD™ FC beads and stained BD™ Stem Cell Control for automated compensation.

Method

The samples were stained using the BD™ Stem Cell Enumeration kit. The stained samples were acquired on the BD FACSLyric™ system using BD FACSuite™ software. The cytometer was set up using BD™ C&T RUO beads for daily performance quality control, BD™ FC beads and stained BD™ Stem Cell Control for automated compensation.

Analysis & Results

Acquired samples were analysed in parallel, using three different methods. They were gated:
A. Manually by BD experts, using BD gating templates in BD FACSuite™ software.
B. Automatically by the new BD FACSYS™ algorithm.
C. Manually by an academic expert, using well-established sequential gating criteria and BD CellQuest™ Pro software.

Each analysis produced cell counts for:
- Viable stem cells (CD34+)
- Total stem cells (CD34+)
- Viable CD45- Cells
- Total CD45+ Cells

The cell counts derived by the three methods were compared using regression analysis.

The matched data show excellent concordance between all three methods (R² = 0.99). Data derived from the algorithm generated slightly lower stem cell counts than BD experts, who in turn generated slightly lower counts than the academic expert. However, the differences were all below 5% for viable and total stem cells, and less than 1% for viable and total CD45+ cells.

Conclusion

The results demonstrate that the BD FACSuite algorithm for the BD Stem Cell Enumeration (SCE) assay on the BD FACSYS system accurately enumerates CD34+ cells.

Legend
- SCE: Stem cell enumeration
- CD45: Cell surface marker