

A Rapid Automated Colony-Forming Cell Assay for Determination of Hematopoietic Progenitors in Cord Blood

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Introduction

The usefulness of the CFC assay for routine evaluation of graft quality and potency could be improved by a shorter assay duration and higher reproducibility of assay results. To achieve these improvements, a semisolid medium (MethoCult[®] Express) was formulated to enable the quantification of total colonies after only 7 days of culture, but without distinction of colony types. In addition, automated read-out of 7 and 14 day assays was achieved with an image-based analysis system specifically designed for colony enumeration. The automated system was used in combination with cultureware designed to improve image quality and colony distribution by eliminating the meniscus at the periphery of the culture dish.

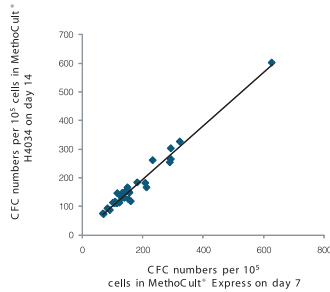
Materials & Method

Umbilical cord blood cells enriched on a Ficoll gradient were inoculated into both MethoCult[®] Express and MethoCult[®] H4034 (STEMCELL Technologies Inc.) and cultured for 7 and 14 days, respectively. Following culture, total colony numbers were determined by manual observation using an inverted light microscope and by imaging and automated analysis using the STEMvision[™] imaging instrument (STEMCELL Technologies Inc., see **Figure 3A**). Variability of assay outcome was assessed by determining the coefficient of variance between multiple manual and automated counts and the correlation between 7 and 14 day assays and between manual and automated counts was assessed by regression analysis.

Results

Total colony counts after 7 days in MethoCult[®] Express were closely correlated with total colonies counted in MethoCult[®] H4034 after 14 days ($r^2=0.97$, $p<0.01$, $n=32$, see **Figure 1**), demonstrating that the shorter assay accurately estimates progenitor numbers in CB samples. In addition, the MethoCult[®] Express assay exhibited greater reproducibility, with a significantly smaller coefficient of variation (CV) between results obtained by multiple assay operators ($6 \pm 3\%$ vs. $11 \pm 5\%$ for MethoCult[®] Express vs. standard assays). The automated colony counts of assays conducted in MethoCult[®] Express were highly correlated to manual microscope counts obtained from a large number of assays conducted ($r^2=0.96$, $p<0.01$, $n=75$, see **Figure 4A**). Likewise, automated total colony counts of 14 day CFC assays conducted in MethoCult[®] H4034 were highly correlated with the average of manual counts conducted by four trained operators ($r^2=0.95$, $p<0.01$, $n=21$, see **Figure 4B**). However, the variability in the manual counts was considerably greater than for the automated system (CV of 13% vs. 4% and 12% vs. 5%, for 7 and 14 day assays conducted in MethoCult[®] Express and MethoCult[®] H4034, respectively). The reduced variability of the automated assay is clearly demonstrated by the narrowing of the confidence intervals around the mean colony counts, as compared to the manual assay (see **Figure 5**).

FIGURE 1: Correlation Between Day 7 CFC Frequencies with MethoCult[®] Express and Day 14 CFC Frequencies with a Standard Medium for 14 Day CFC Assays, MethoCult[®] H4034



7 day CFC numbers in MethoCult[®] Express are significantly correlated with 14 day CFC numbers in standard MethoCult[®] H4034 ($R^2=0.97$, Slope=0.94, $P<0.01$, $n=32$).

FIGURE 2: Composite STEMvision[™] Images

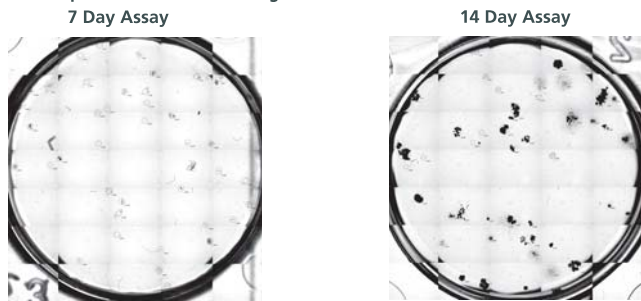
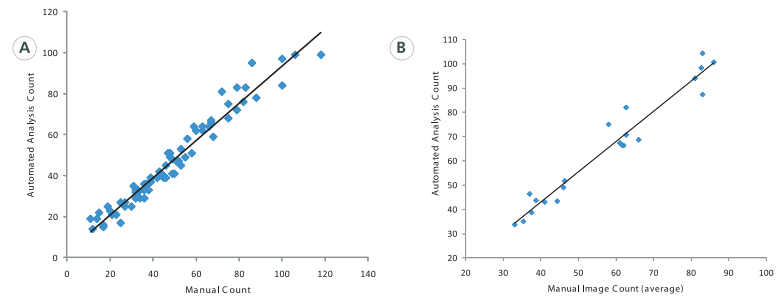


FIGURE 3: STEMvision[™] Fully Automated CFC Assay Reader (A), Colony Image in Regular Dish (B) vs. Meniscus-Free SmartDish (C)

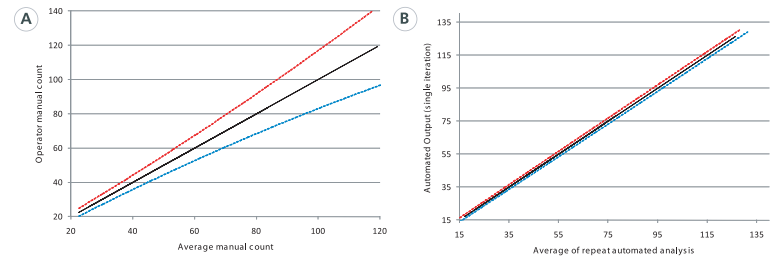


FIGURE 4: Correlation Between Manual Counts and STEMvision[™] Automated Analysis



Analysis of CFC assay cultures at day 7 of **A**) MethoCult[®] Express and day 14 of **B**) MethoCult[®] H4034 using STEMvision[™] automated colony counting and manual counting by light microscopy. There is a high degree of correlation between the manual and automated methods. Regression analysis demonstrates significant correlations ($r^2=0.96$, slope=1.05, $P<0.01$ for assays in MethoCult[®] Express; and $r^2=0.95$, slope=1.2, $P<0.01$ for assays in MethoCult[®] H4034).

FIGURE 5: Variability of STEMvision[™] Automated Colony Analysis vs. Manual Counting



A) Manual microscope analysis and **B**) automated analysis of 7 day CFC assay cultures conducted in MethoCult[®] Express. The 95% confidence intervals over a range of colony counts are shown for replicate manual and automated analyses of the same culture dishes. With manual counting, the confidence interval diverges with the total colony number counted per plate, whereas with STEMvision[™] analysis the confidence interval is uniform through the typical range of colony densities and smaller than when counting manually. Similar results were obtained with the 14 day CFC assay.

Conclusions

- MethoCult[®] Express provides a shortened CFC assay with high reproducibility for counting total colonies derived from hematopoietic progenitors in CB.
- Automated quantification of total colonies for both the 7 day and 14 day CFC assays using the STEMvision[™] instrument is a reliable substitute for microscopic counting.
- Colony counting with the automated system reduces counting variability and provides a new method for standardizing the CFC assay within and between CB banks and cell therapy labs.