

Standardization and Automation of the Colony-Forming Cell Assay for Measuring Hematopoietic Progenitors in Cord Blood

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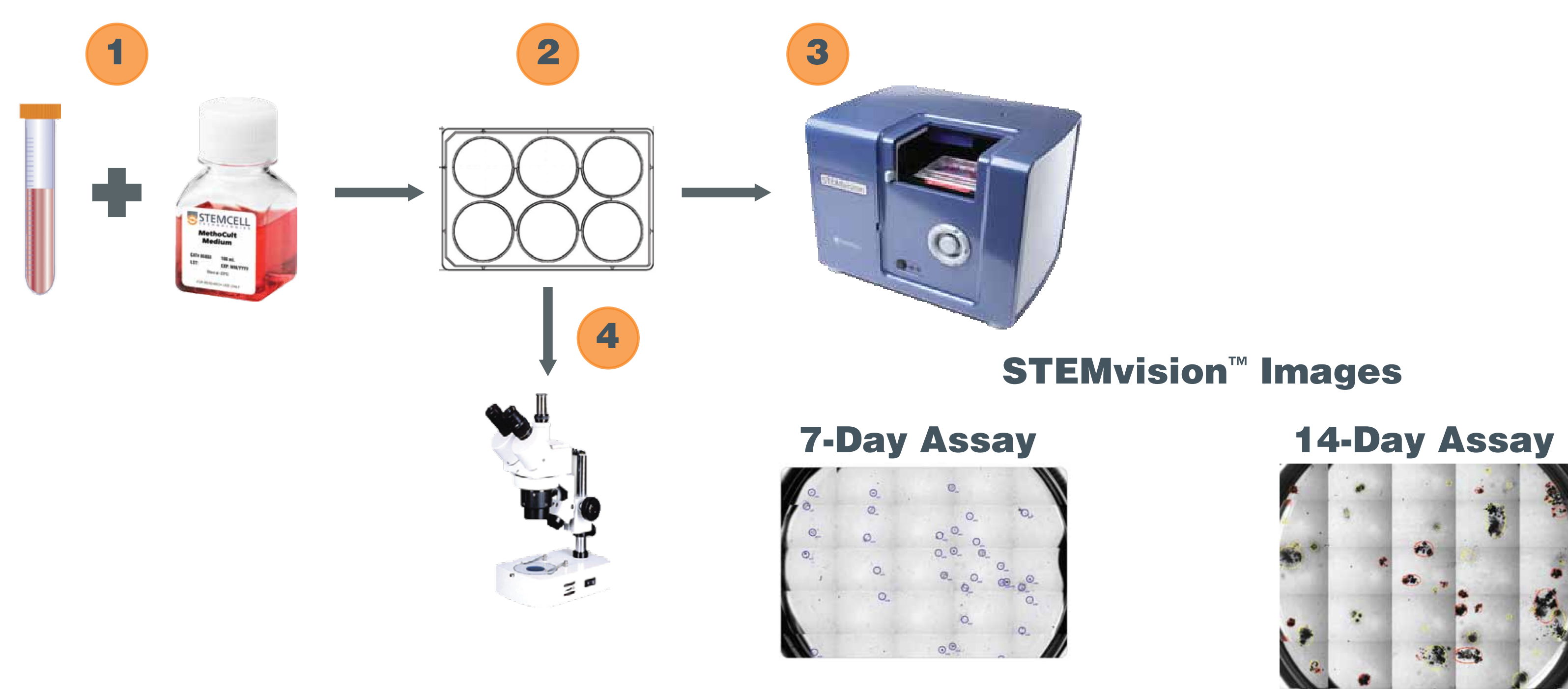
Introduction

The colony forming cell (CFC) assay is the standard functional assay for measuring the hematopoietic progenitor content of cord blood (CB) and other cell products for hematopoietic stem cell transplantation (HSCT). The CFC content of CB is a strong predictor of neutrophil and platelet engraftment after unrelated CB Transplantation (Blood. 2008 Oct 1; 112(7):2979-89; Biol Blood Marrow Transplant. 2011 Jan 28 [Epub ahead of print]). Automation of colony enumeration and shorter assay duration would facilitate the use of the CFC assay for evaluation of graft quality for CB banking, graft selection, and quality control of cell processing procedures.

We have developed an imaging and analysis instrument (STEMvision™) for automated colony enumeration and classification in CFC assays on human CB samples. Two CFC assays formats were tested:

1. A novel 7-day assay in MethoCult® Express medium for detecting total progenitors in CB without distinction of colony types.
2. The standard 14-day assay in MethoCult® H4034 Optimum medium for enumerating erythroid, myeloid and multi-lineage colonies.

FIGURE 1: The hematopoietic CFC assay



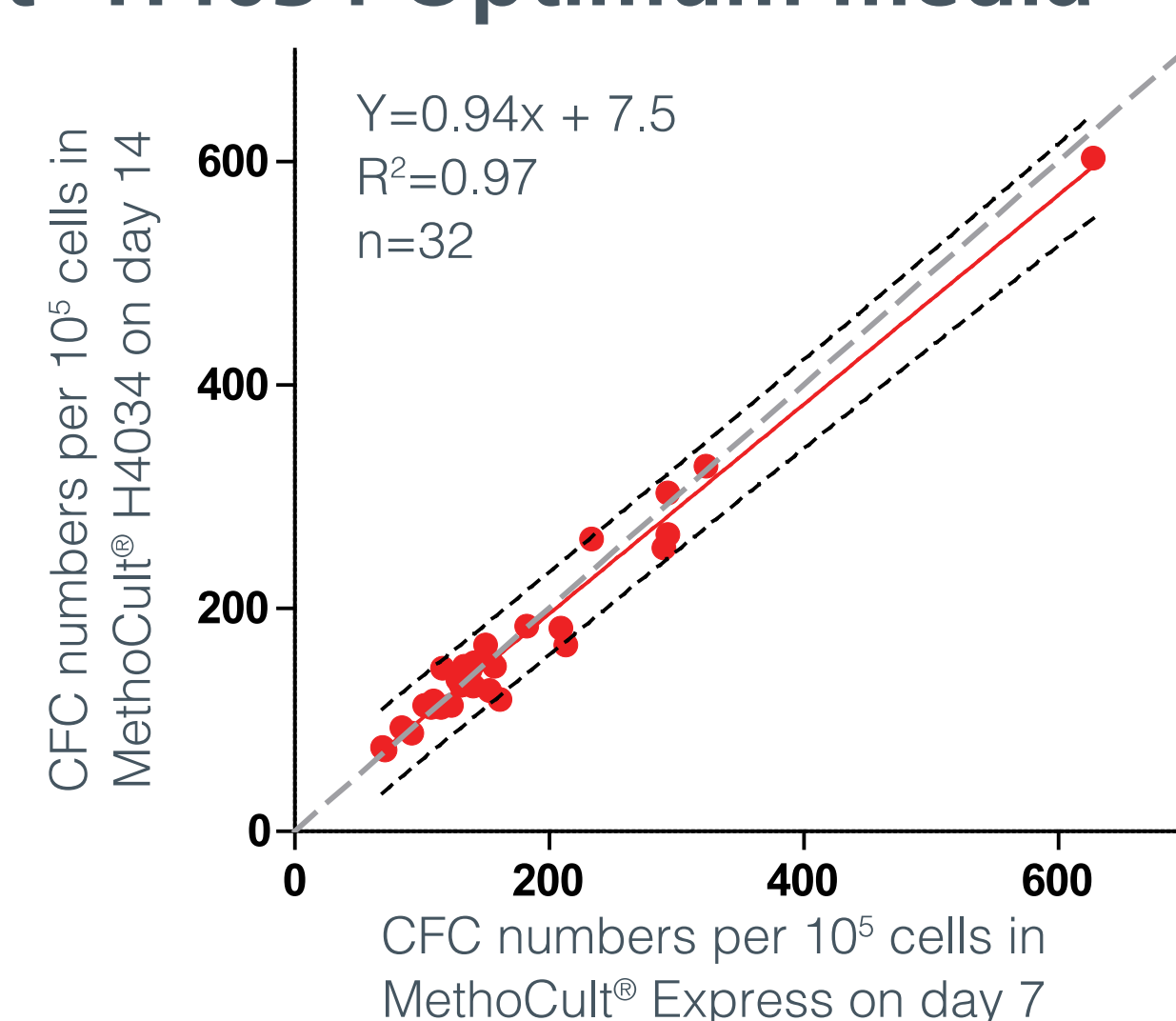
1. A sample of cord blood cells is added to MethoCult® Express (7-day assay) or MethoCult® H4034 Optimum (14-day assay) medium.
2. The medium is plated in 35mm 6-well SmartDish™ low-meniscus cultureware.
3. Colonies are counted automatically using the STEMvision™ instrument and software.
4. Colonies are counted manually by four experienced operators using a standard inverted light microscope.

Study Objectives

1. Examine the relation between results of 7-day CFC assays in MethoCult® Express and of 14-day CFC assays in MethoCult® H4034 Optimum (**Figure 2**).
2. Examine the relation between manual counts and automated colony assay results in 7-day assays for total CFCs and in 14-day assays for total, erythroid, and myeloid (plus multi-lineage) CFCs (**Figure 3**).
3. Examine the reproducibility of manual and automated counting methods (**Figure 4**).

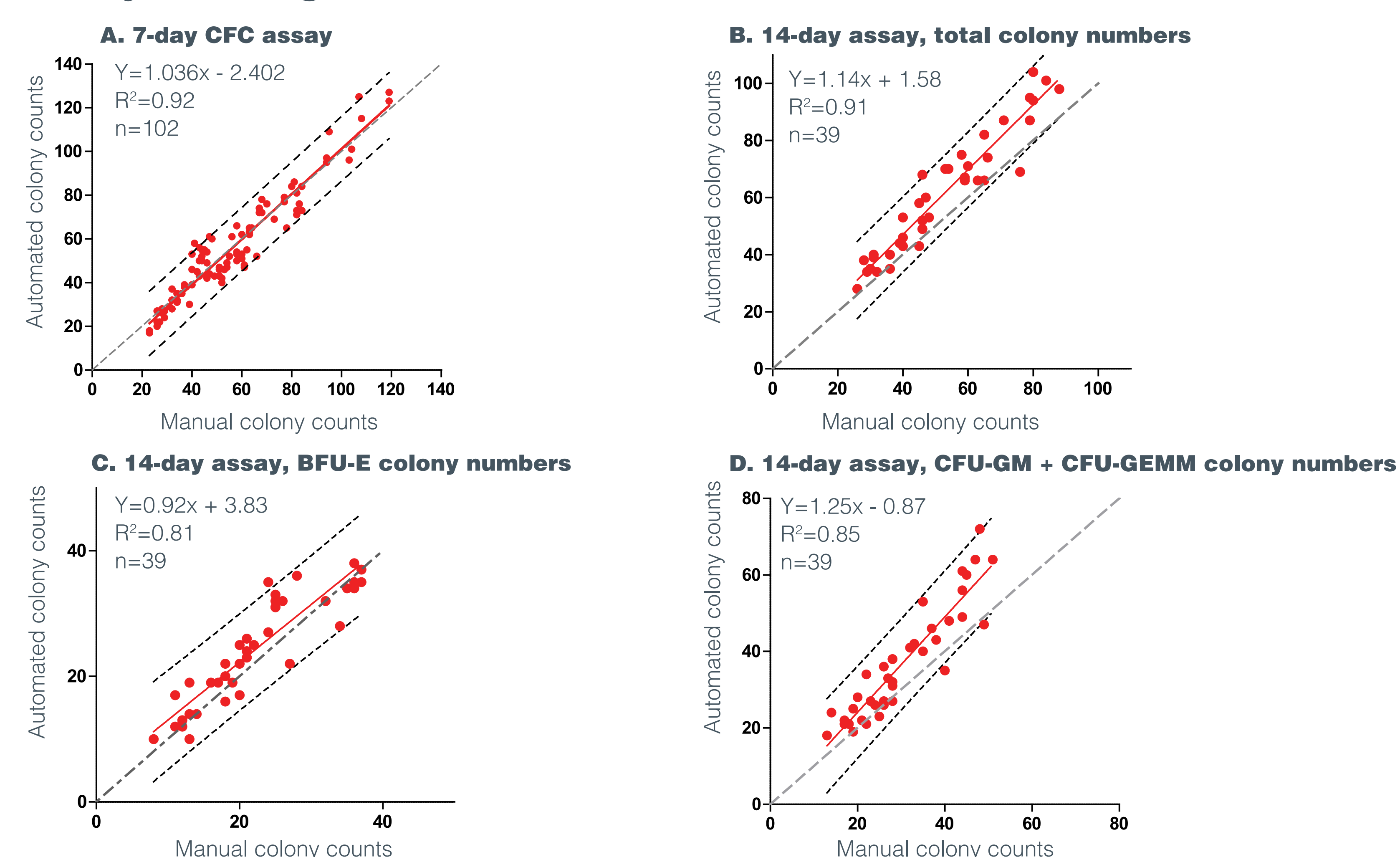
Results

FIGURE 2: Correlation between CFC frequencies in cord blood obtained in 7-day CFC assays using MethoCult® Express and 14-day CFC assays using MethoCult® H4034 Optimum media



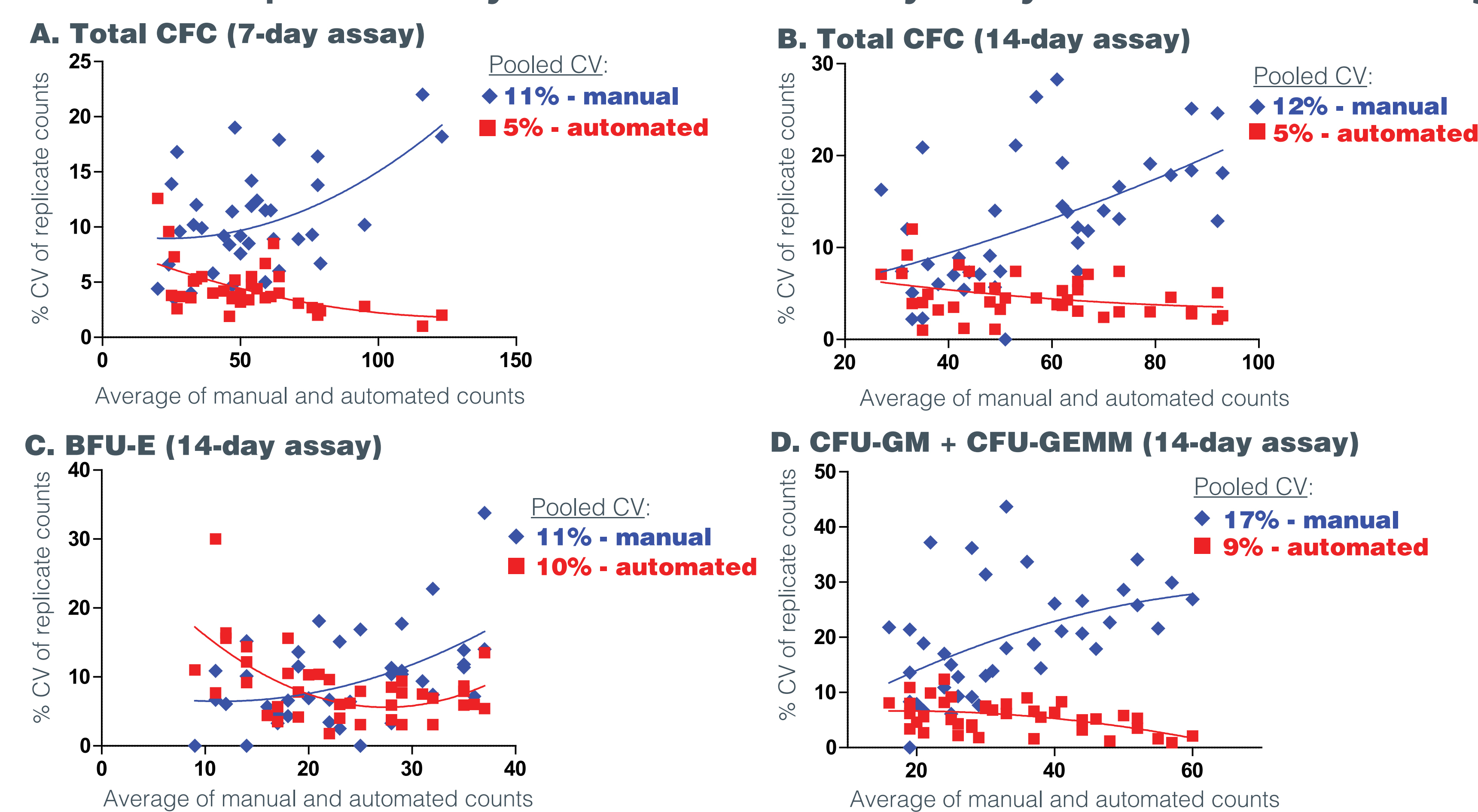
Total CFC frequencies measured by manual counting of 7-day CFC assays in MethoCult® Express and 14-day assays in MethoCult® H4034 are strongly related ($p < 0.001$). This demonstrates that the 7-day CFC assay can enumerate total progenitors in CB as accurately as the 14-day assay, but without distinction of colony types. The black dashed lines demarcate the 95% prediction limits, i.e., the area in which 95% of data points are expected to fall. The gray dashed line represents the ideal correlation when both assays give identical results ($y=x$).

FIGURE 3: Correlation between manual and STEMvision™ automated colony counting



Analysis of CFC frequencies in 7-day CFC assays in MethoCult® Express (**A**, total CFC) and 14-day CFC assays in MethoCult® H4034 Optimum (**B**, total CFC; **C**, BFU-E; **D**, CFU-GM+CFU-GEMM) using STEMvision™ automated colony counting and manual counting by three to four experienced operators. The black dashed line in each graph demarcates the 95% prediction limits, i.e., the area in which 95% of data points are expected to fall. The gray dashed line represents the ideal correlation when both assays give identical results ($y=x$).

FIGURE 4: Reproducibility of automated colony analysis vs. manual counting



The variability of replicate colony counts, expressed as coefficient of variation (CV), was plotted against the average colony counts for individual culture wells. The results show the variability of replicate manual counts by four experienced operators (**blue diamonds**) and between replicate automated counts obtained by repeated imaging the same cultures on four STEMvision™ instruments (**red squares**) in 7-day CFC assays (**A**, total CFC) and 14-day CFC assays (**B**, total CFC; **C**, BFU-E; **D**, CFU-GM + CFU-GEMM), respectively. The variability between replicate manual colony counts of individual cultures increased with the number of colonies per dish. In comparison, the variability between replicate automated counts was less affected by colony counts and tended to be lower in cultures with many colonies than in cultures with few colonies. The overall variability (pooled CV) of replicate STEMvision™ counts (5-10%) was 2-fold lower than the overall variability of the manual counts (11-17%), except for BFU-E counts which showed similar variability between replicate STEMvision™ and manual counts (i.e. ~10%).

Conclusions

- CFC assays in MethoCult® Express provide accurate information about the total hematopoietic progenitor content of CB, but without distinction of colony types, and can be completed in only 7 days as compared to the 14 days of standard CFC-assays.
- Automated STEMvision™ identification and enumeration of colonies in 7 and 14-day CFC assays is a reliable substitute for manual colony counting.
- Automated STEMvision™ colony scoring is at least as reproducible as manual scoring by experienced operators and can be expected to increase the reproducibility and consistency of CFC assay results both within and between laboratories.
- Future optimization of STEMvision™ is expected to further increase the correlation and agreement between manual and automated CFC assay results for erythroid and myeloid progenitors, and to extend automated colony scoring to CFC assays on peripheral blood, bone marrow, and other cells.