STEMvision™

Automated and Standardized Counting of CFU Assays for Cord Blood Banks

STEMCELL™ TECHNOLOGIES

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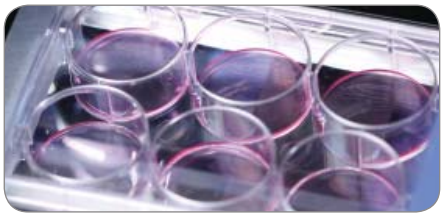
A Complete Set of Tools for the CFU Assay

STEMCELL Technologies, Inc. offers a comprehensive line of products to determine the number of CFUs in cord blood samples. STEMCELL Technologies' Quality Management System is certified to ISO 13485 Medical Device Standards.

Please visit us at **www.stemcell.com** for additional information.

The Colony-Forming Unit (CFU) Assay for

Cord Blood Banks




The colony-forming unit (CFU) assay is the gold standard in vitro functional assay for identifying and counting hematopoietic progenitor cells. It can be used by cord blood (CB) banks to evaluate the viability and CFU content of cell products.

The utility of the CFU assay for CB stem cell transplantation has been demonstrated by numerous clinical studies involving patients with a variety of hematological and non-hematological cancers and other disorders. These studies show that the number of CFUs in a CB unit, especially in cryopreserved cells thawed for unrelated transplantation, is the one parameter that best correlates with the time to neutrophil and platelet engraftment, and overall survival following CB transplantation.[1-5]

Based on these studies, several regulatory bodies (e.g. FDA, AABB, NetCord-FACT) recommend or require that the number of CFUs in a CB unit be measured before cryopreservation and/or after thawing, prior to release of the unit to a transplant facility. STEMCELL Technologies offers a complete set of tools for CB banks to determine the number of CFUs in CB samples (Figure 1).

A Complete Workflow for Cord Blood CFU Assays:

- HetaSep™ for removing red blood cells from fresh CB samples
- MethoCult™ Express and MethoCult™ Optimum media for 7- and 14-day CFU assays, respectively
- SmartDish™ meniscus-free cultureware for more accurate CFU counting
- STEMvision™ for automated and standardized counting of BFU-E, CFU-G/M/GM and CFU-GEMM
- Analysis Packages for counting 7-day and 14-day CB CFU assays
- Proficiency testing programs

Public Cord Blood Banks

The CFU assay can be used to identify CB units containing a high number of progenitor cells. Pre-selection of CB units with a high CFU content ensures that valuable space and resources are expended only on banking the most promising units, and thereby improves the quality of the CB repository.

The CFU assay can also be used to determine whether processing and cryopreservation have adversely affected the number of viable progenitor cells in a CB unit. This ensures that the procedures used in individual laboratories yield CB products with high hematopoietic potential.

Finally, the CFU assay can be used to assist in selecting one or more suitable CB unit(s) for transplantation. The number of viable and functional CFUs in a CB unit after thawing is a key determinant guiding selection of units with high hematopoietic potential.

Private Cord Blood Banks

The CFU assay provides families with important biological information about the quality of their child's CB sample at the time of collection. It can provide parents with assurance that the hematopoietic progenitor cells in their child's CB are viable in the event that the unit is needed for hematopoietic rescue later in life. This information can also help parents make their financial investment with greater confidence.

The benefits of the CFU assay can be highlighted to families in the process of deciding which bank to use to process and store their CB cells. It offers a means for private CB banks to differentiate themselves from their many competitors in the marketplace.

The CFU Assay Workflow

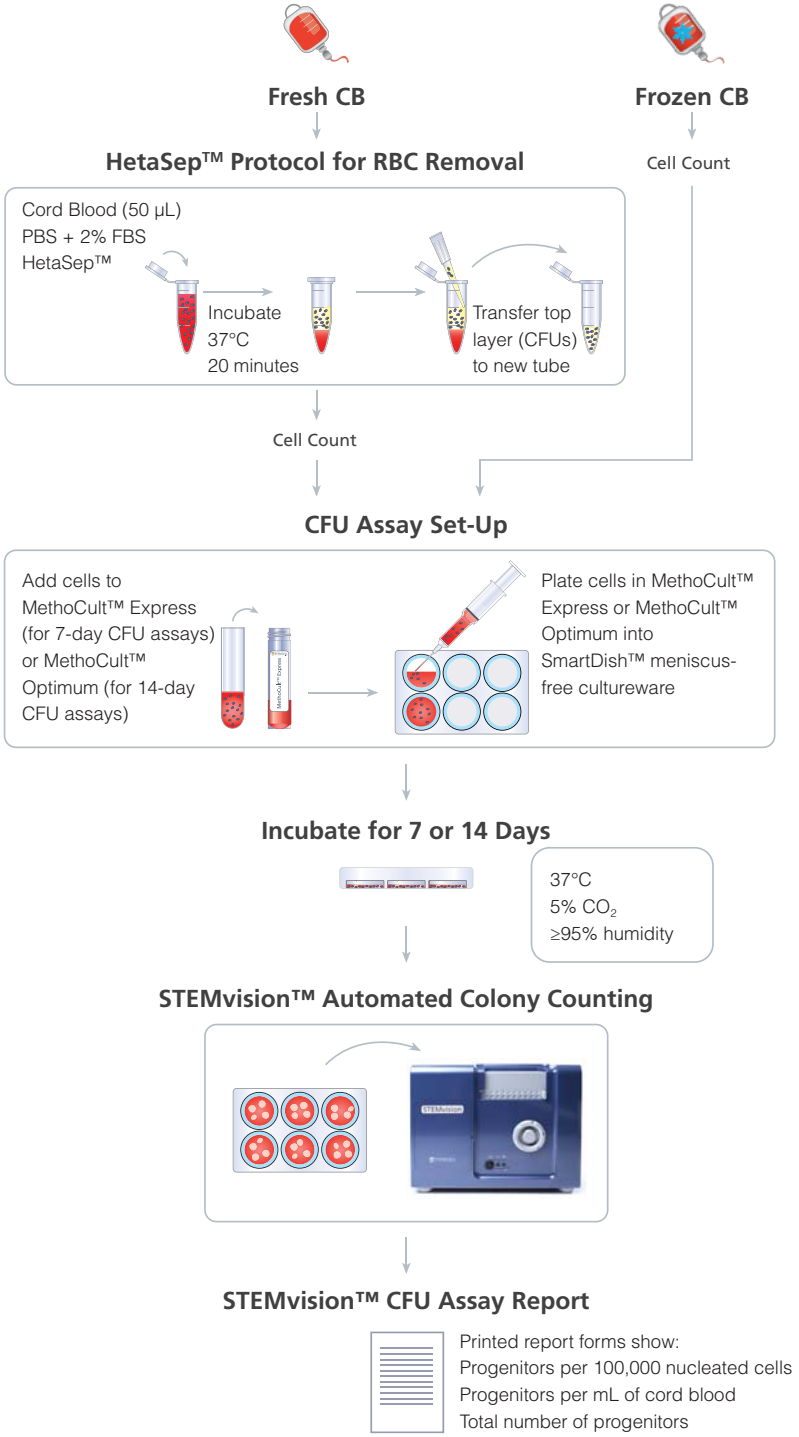


Figure 1. A Typical CFU Assay Workflow for a CB Bank With STEMvision™ Integrated for Automated Counting of Hematopoietic Colonies

Red blood cells (RBCs) are removed from a 50 µL sample of fresh cord blood (CB) using HetaSep™. This step is not required for thawed cryopreserved CB cells. Fresh or thawed CB cells are then cultured in the appropriate MethoCult™ medium, depending on whether the different colony-forming unit (CFU) subtypes will be scored after 14-days (MethoCult™ Optimum) or if total CFU numbers will be counted after 7-days (MethoCult™ Express). STEMvision™ is used to acquire an image of the culture dish and analyze the image to identify and enumerate the different colony types. The results of the CFU assay can be documented in the form of a printed report showing the frequency and total number of CFUs in the CB unit. These forms are available in two formats; one for the lab and another for the family (if desired).

STEMvision™

Automated Imaging and Standardized Colony Counting

STEMvision™ is a bench-top instrument and computer system that automates and standardizes the process of counting hematopoietic colonies in the colony-forming unit (CFU) assay. STEMvision™ images each 35 mm well in approximately 1 minute, resulting in a high-resolution image. With our updated color instrument, colonies containing hemoglobinized cells are shown in their true red color. Sophisticated analysis software is then used to identify, classify and count the colonies produced by BFU-E, CFU-G/M/GM and CFU-GEMM progenitors, in approximately 1 minute per well (Figure 2).

By using an automated system to standardize colony identification and counting, cord blood (CB) banks can ensure that their CFU assay results are accurate and reproducible. STEMvision™ Analysis Packages have been developed to provide total CFU counts and colony classification in the conventional 14-day assay, or total CFU counts only in a faster 7-day assay of human CB cells. For detailed product information please see page 14.

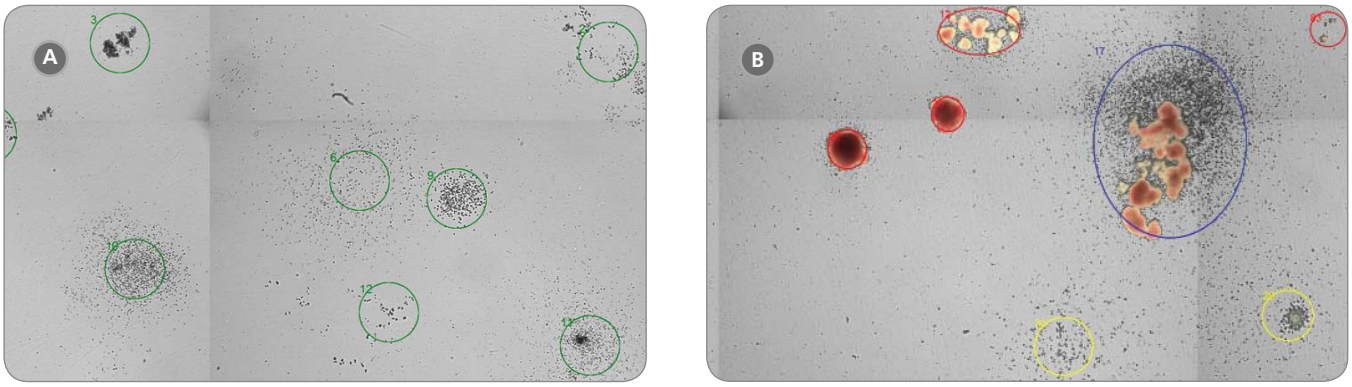


Figure 2. Representative STEMvision™ Images Showing Colonies Derived from CB Progenitors After 7 Days of Culture in MethoCult™ Express or 14 Days of Culture in MethoCult™ Optimum

The images have been analyzed with the STEMvision™ Human CB (A) 7-Day and (B) 14-Day Analysis Packages. Green circles identify individual colonies in the 7-day CB CFU assay that counts the total number of CFUs only (A). Red circles identify erythroid colonies (produced by BFU-E), yellow circles identify myeloid colonies (produced by CFU-G, CFU-M or CFU-GM) and blue circles identify mixed colonies (produced by CFU-GEMM) in the 14-day CB CFU assay (B). Erythroid and mixed colonies that contain hemoglobinized cells are shown in true red color.

STEMvision™ Can Help You:

- Automate and standardize colony counting to ensure accurate and reproducible CFU assay results
- Minimize intra- and inter-individual and laboratory variation in colony counting
- Digitally image cultures for permanent record keeping
- Collect and review data in an easy to use format
- Save time in staff training and laboratory workflows

STEMvision™

Performance Data

The total number of hematopoietic colonies and the numbers of erythroid, myeloid and mixed colony sub-types in colony-forming unit (CFU) assays of cord blood (CB) cells counted using STEMvision™ is highly correlated with the number of colonies counted manually using an inverted microscope. Importantly, colony counts produced by STEMvision™ show significantly lower variability in the recommended range of 20-80 colonies per 35 mm culture well than colony counts produced manually by multiple technicians scoring the same CFU assays.

The correlation between automated and manual colony counting, and the reduced variability observed with automated CFU counting are shown in Figures 3-5.

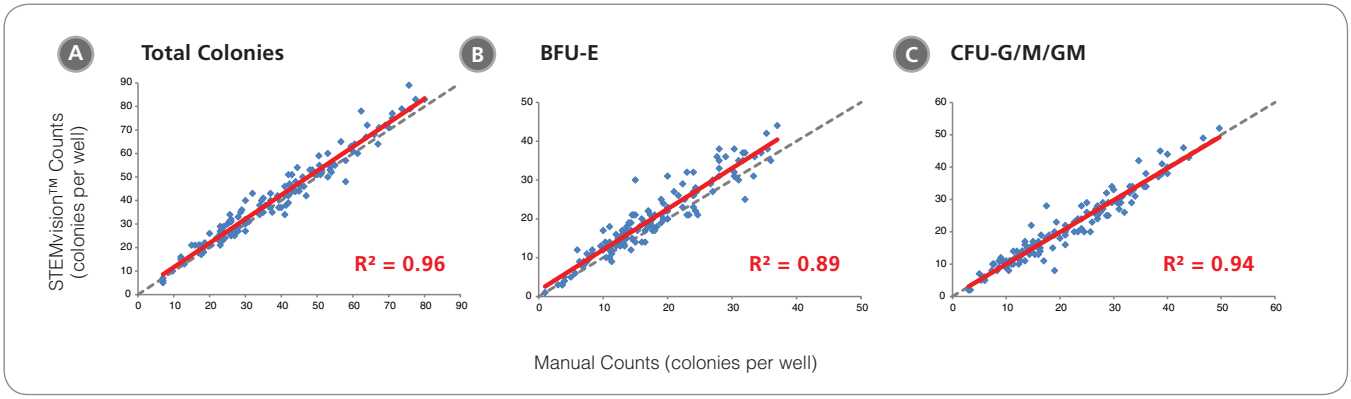


Figure 3. STEMvision™ Automated Counts of Total, Erythroid (BFU-E) and Myeloid (CFU-G/M/GM) Colonies are Highly Correlated to Manual Counts of 14-Day CB CFU Assays

Cryopreserved CB samples were thawed, plated in MethoCult™ Optimum, cultured for 14 days and scored both manually using an inverted microscope and automatically using STEMvision™. The results show a strong correlation between automated counts using STEMvision™ and manual counts. Gray dashed lines represent a perfect linear correlation between manual and automated counts. Red solid lines represent the actual linear correlation between manual and automated counts.

The mathematical equations and correlation coefficients (R^2) that describe each data set (n=130 CFU assays) are as follows:

Figure 3A: $y=1.02x + 1.39$; $R^2=0.96$ for Total Colonies

Figure 3B: $y=1.05x + 1.53$; $R^2=0.89$ for BFU-E

Figure 3C: $y=0.99x + 0.13$; $R^2=0.94$ for CFU-G/M/GM

Performance Data

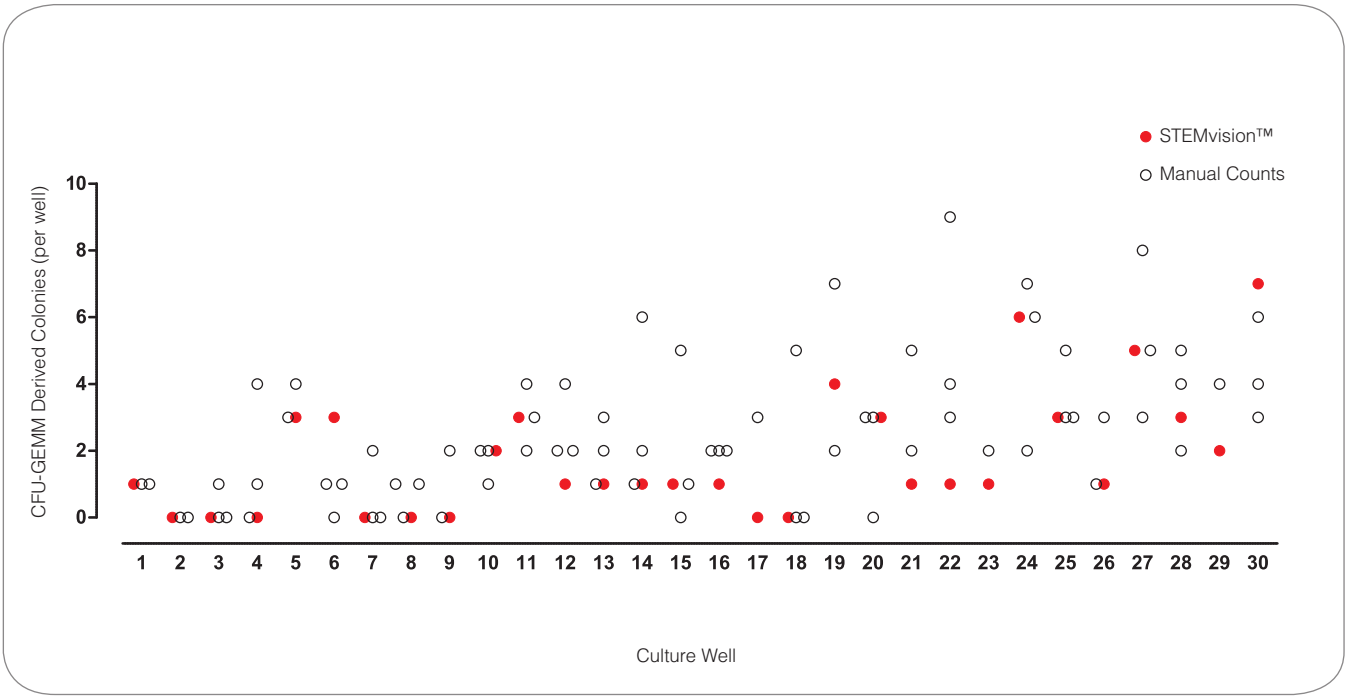


Figure 4. STEMvision™ Automated Counting of Mixed Colonies Falls Within the Range of Manual Counts of 14-Day CB CFU Assays

Thirty individual 14-day CB CFU assays were counted by three to seven people. The numbers of mixed (CFU-GEMM) colonies counted manually in each well are shown as open circles (n=80 total assay scores). Manual CFU-GEMM counts in most cultures varied between individual people. STEMvision™ counts of the same culture wells (red circles) provided a CFU-GEMM count that was typically within the range of manual counts.

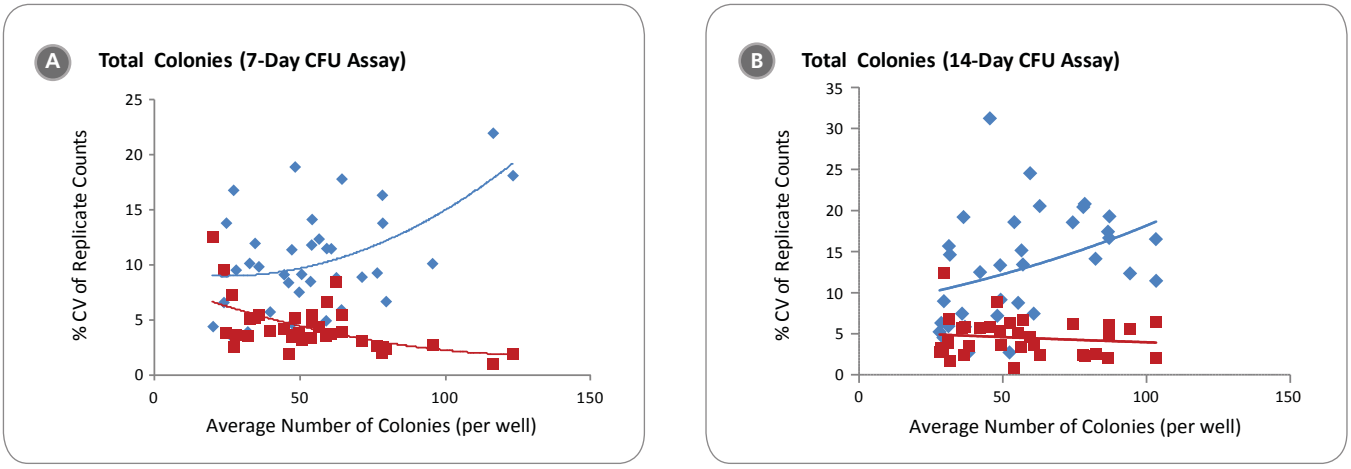


Figure 5. STEMvision™ Automated Colony Counting of 7-Day and 14-Day CB CFU Assays is More Reproducible Than Manual Counting

The coefficients of variation (CV) for total colony counts in (A) 7-day and (B) 14-day CFU assays of CB cells were determined by counting the same culture wells either manually by three to five different people (blue diamonds), or automatically using three to five separate STEMvision™ instruments (red squares). The average CVs for 7-day and 14-day total colony counts produced manually were 11% and 13%, respectively. CVs for 7-day and 14-day colony counts produced by STEMvision™ were 5%.

STEMvision™

CFU Assay Report Forms

STEMvision™ produces two printed reports that detail information about the specific cord blood (CB) unit and the colony-forming unit (CFU) assay results (Figure 6). These reports provide critical functional information about the CB sample for the bank's own records and for the family (if desired). The values documented in these reports include:

- CB bank address and contact information
- Patient and doctor demographic information
- CB sample and CFU assay tracking ID numbers
- Number of viable progenitor cells per 100,000 nucleated cells
- Number of viable progenitor cells per mL of CB
- Total number of viable progenitor cells in the CB unit
- Counts for BFU-E, CFU-G/M/GM and CFU-GEMM are shown separately on 14-day CFU assay report forms
- Images of each replicate CFU assay displaying colonies and their classifications (colored circles)

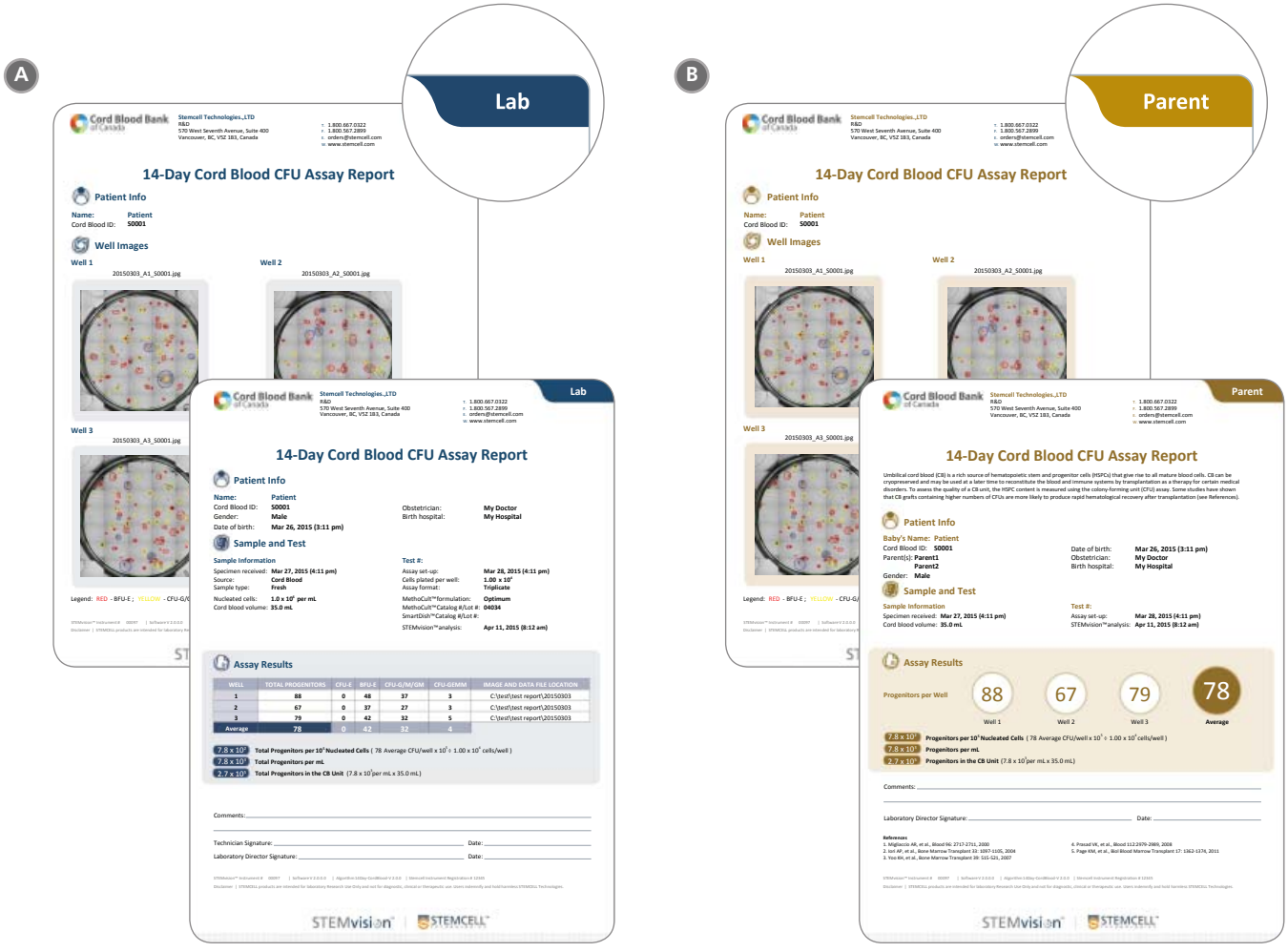


Figure 6. Sample STEMvision™ Lab and Parent Reports for a 14-Day Cord Blood CFU Assay

Printed reports can be generated in two versions, as both (A) Lab, for the CB bank's own records, and (B) Parent, for the family, if desired.

HetaSep™
for Red Blood Cell Removal

Benefits of HetaSep™:

- Increases the accuracy of colony counting
- Greater than 97% recovery of CFUs
- Fast and easy to perform
- Requires only 50 µL of blood

HetaSep™

PRODUCT:	HetaSep™	
CATALOG #:	07806	20 mL/bottle
	07906	100 mL/bottle

Large numbers of red blood cells (RBCs) in a colony-forming unit (CFU) assay prevent hematopoietic colonies from being accurately visualized either manually or using STEMvision™ (see Figure 7). RBCs must therefore be removed from fresh CB samples (whether whole or processed) before performing the CFU assay. RBCs do not need to be removed from cryopreserved samples as only a low proportion of RBCs survive freezing and thawing.

HetaSep™ is an erythrocyte aggregation agent used to quickly separate nucleated cells from RBCs. It is based on the principle that aggregated erythrocytes settle much faster than dispersed cells.

The HetaSep™ procedure does not affect the number of progenitor cells; 97% of CFUs are recovered in the RBC cleared sample. HetaSep™-mediated RBC depletion requires only 50 µL of CB and is quick, making it easy to incorporate into an institution's workflow.

See the HetaSep™ Protocol Technical Bulletin (Document #29541) for more information: **www.stemcell.com/hetasep_protocol**.

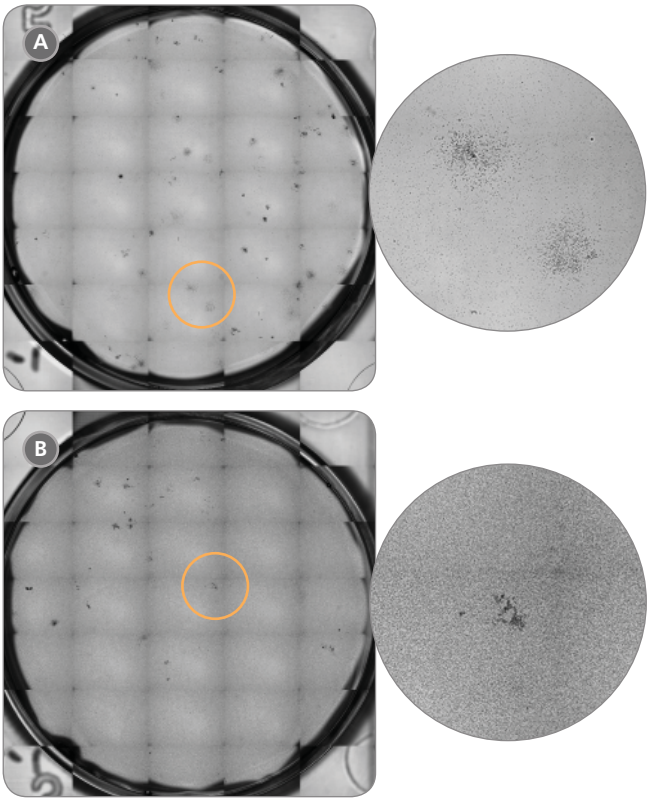


Figure 7. STEMvision™ Images of 7-Day CFU Assays of Fresh Cord Blood Samples Plated in MethoCult™ Express With and Without Prior Removal of RBCs Using HetaSep™

(A) Acceptable background (minimal RBCs) for CFU assay with prior HetaSep™ treatment. (B) Unacceptable background for CFU assay without prior HetaSep™ treatment. Note that fewer colonies are visible due to increased RBC background.

SmartDish™

Meniscus-Free Cultureware for More Accurate Counting of CFU Assays

When a hematopoietic colony-forming unit (CFU) assay is performed using traditional cultureware, a meniscus is formed between the culture medium and the sides of the culture dish. This meniscus results in an increased medium depth at the periphery of the dish, leading to a higher proportion of colonies forming along its edges. Shadows and optical distortion caused by the meniscus can make colony identification more challenging at the edges of the dish (Figure 8A), reducing accuracy through undercounting of CFUs.

SmartDish™ 6-well culture plates have been designed to enable accurate and reproducible colony counting by preventing the formation of a meniscus. This allows for an even distribution of culture medium, resulting in a more uniform distribution of colonies throughout the entire well. The absence of a meniscus reduces optical distortion so that colonies located at the edge of each well can be more easily counted (Figure 8B). SmartDish™ cultureware has been designed to work in conjunction with STEMvision™ for automated counting of hematopoietic CFU assays, and is required to provide accurate and reproducible colony counting results.

Benefits of SmartDish™*:

- Even distribution of colonies throughout each well
- No shadow or optical distortion at well edges
- Easier colony counting
- Increased colony counting accuracy

SmartDish™ Meniscus-Free Cultureware

PRODUCT:	SmartDish™ (6-well plates)	
CATALOG #:	27301	5/pack
	27302	50/pack

REQUIRED FOR:

- Easier and more accurate colony counting in the hematopoietic CFU assay
- Automated colony counting with STEMvision™

*Patent pending

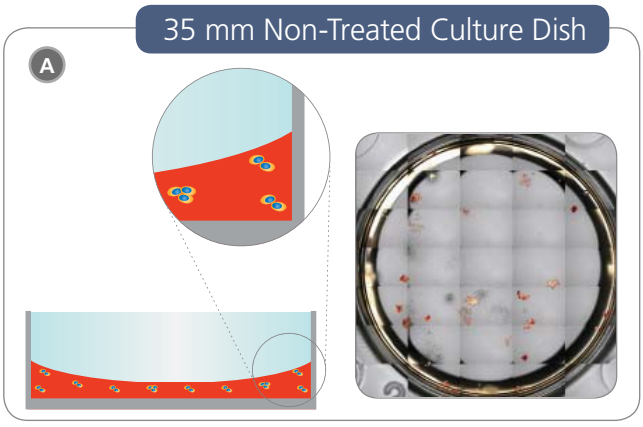


Figure 8. 14-Day CB CFU Assays Performed in Standard Non-Treated and SmartDish™ 6-Well Culture Plates.

Shown are representative STEMvision™ images of 35 mm wells from either a (A) non-treated culture dish or (B) SmartDish™. The formation of a meniscus in standard cultureware causes more colonies to form around the periphery of the dish where the culture medium is deeper (A). Optical distortion obscures these colonies and makes them more difficult to count. Colonies are easier to count at the edge of the SmartDish™, which has been treated to eliminate the meniscus, allowing a more equal distribution of colonies (B).

MethoCult™ Optimum

Methylcellulose Medium for 14-Day CFU Assays

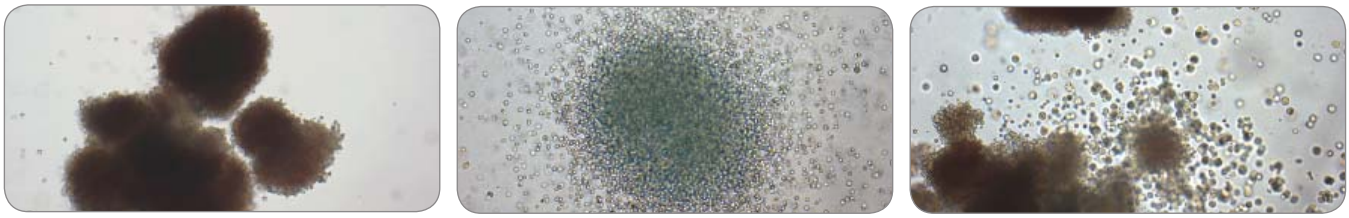


Figure 9. Microscope Images of Colonies Derived From BFU-E, CFU-GM and CFU-GEMM Progenitors, Respectively, After 14-Day Cultures in MethoCult™ Optimum

The STEMvision™ Human Cord Blood 14-Day Analysis Package has been designed for use with MethoCult™ Optimum medium (Catalog #04034/04044). This medium is considered to be the gold standard in the hematopoiesis field and is used extensively in global proficiency testing programs. MethoCult™ Optimum is formulated to support optimal proliferation and differentiation of BFU-E, CFU-G/M/GM and CFU-GEMM. For more details, please visit **www.stemcell.com/CFUwallchart**.

MethoCult™ Optimum is CE Marked for in vitro diagnostic (IVD) use in the European Union (EU). The CE Mark indicates that this medium complies with EU safety, environmental and quality standards required for IVD medical devices. It complies with Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Diagnostic Medical Devices within applicable countries.

Outside of the EU, MethoCult™ Optimum is for research use only unless otherwise determined, not for therapeutic or diagnostic use.

Benefits of MethoCult™ Optimum:

- Formulated for use with cord blood, isolated $CD34^+$ cells and other tissues
- Optimized for a wide range of applications
- Available in formulations with and without EPO

TECHNICAL BULLETIN

"Potency" Assays for Measuring the Engraftment Potential of Hematopoietic Stem and Progenitor Cells

www.stemcell.com/potency_assay

Table 1. MethoCult™ Optimum Media Currently Validated for Automated Counting With STEMvision™**

METHOCULT™ PRODUCT	CATALOG #	SIZE	COMPONENTS				APPLICATIONS
			MC	FBS	BSA	GROWTH FACTORS	
MethoCult™ Optimum	04034/84434* 04044/84444*	100 mL 24 x 3 mL	✓	✓	✓	Cytokines, including erythropoietin (EPO)	Supports growth of CFU-E, BFU-E, CFU-G/M/GM and CFU-GEMM in human CB

MC: methylcellulose; FBS: fetal bovine serum; BSA: bovine serum albumin; CB: cord blood

*CE Marked for IVD use in the EU.

**Please contact Tech Support for more information.

MethoCult™ Express

Methylcellulose Medium for 7-Day CFU Assays

Figure 10. Hematopoietic Colonies Grown in MethoCult™ Express, Visualized on Day 7 (Left) and Day 14 (Right) Under a Microscope

Historically, human colony-forming unit (CFU) assays have been counted after 14 days. This period allows sufficient time for progenitor cells to differentiate into the different hematopoietic lineages so that the various sub-types of CFUs can be scored separately. While these 14-day assays are preferred by many labs, they are not essential to simply measure the total number of viable and functional CFUs.

The STEMvision™ Human Cord Blood 7-Day Analysis Package is designed to be used with MethoCult™ Express (Catalog #04437/04447). This medium is formulated to accelerate the proliferation of hematopoietic progenitor cells so that colonies can be counted after only 7 days; one week faster than with a conventional 14-day CFU assay. This approach provides a simple method to more quickly determine the total number of viable and functional progenitor cells in a cord blood (CB) unit. The total number of CFUs in a CB unit has been shown by several clinical studies to strongly correlate with engraftment outcomes following CB transplantation.[1-5]

Benefits of MethoCult™ Express:

- Formulated for accelerated progenitor cell proliferation and colony formation
- Allows counting of total CFU numbers from CB samples in only 7 days

The total number of CFUs in CB measured after 7 days of culture in MethoCult™ Express correlates strongly with total CFU numbers measured after 14 days of culture in MethoCult™ Optimum (Catalog #04034/04044), or in other formulations, including MethoCult™ Classic (Catalog #04434/04444) and MethoCult™ Enriched (Catalog #04435/04445).

MethoCult™ Express is CE Marked for in vitro diagnostic (IVD) use in the European Union (EU). The CE Mark indicates that this medium complies with EU safety, environmental and quality standards required for IVD medical devices. It complies with Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Diagnostic Medical Devices within applicable countries.

Outside of the EU, MethoCult™ Express is for research use only unless otherwise determined, not for therapeutic or diagnostic use.

Table 2. MethoCult™ Express Media Currently Validated for Automated Counting with STEMvision™**

METHOCULT™ PRODUCT	CATALOG #	SIZE	COMPONENTS				APPLICATIONS
			MC	FBS	BSA	GROWTH FACTORS	
MethoCult™ Express	04437* 04447*	100 mL 24 x 3 mL	✓	✓	✓	Cytokines, including erythropoietin (EPO)	Measures the total number of CFUs in cord blood in only 7 days

MC: methylcellulose; FBS: fetal bovine serum; BSA: bovine serum albumin
*CE Marked for IVD use in the EU.
**Please contact Tech Support for more information.

STEMvision™

Product Information

PRODUCT:	STEMvision™ Instrument
CATALOG #:	22000/22000E

PRODUCT:	STEMvision™ Human Cord Blood 7-Day CFU Analysis Package
CATALOG #:	22001

PRODUCT:	STEMvision™ Human Cord Blood 14-Day CFU Analysis Package
CATALOG #:	22005

SYSTEM IS SUPPLIED WITH:

- STEMvision™ base unit (#22102C)
- Computer and monitor (#22101)
- Software for image acquisition, analysis and review (Catalog #22001 or #22005 as selected above)
- One- or two-year warranty

REQUIRED REAGENTS:

- HetaSep™ (page 10)
- SmartDish™ cultureware (page 11)
- MethoCult™ Optimum (page 12)
- MethoCult™ Express (page 13)

CAPACITY:

- One 6-well SmartDish™ at a time
- Imaging each individual well of a 6-well SmartDish™ takes approximately 1 minute
- Image analysis takes approximately 1 minute per well but can be performed at a later time

DIMENSIONS:

- 478 mm W x 335 mm D x 347 mm H
- 18.82 in W x 13.19 in D x 13.66 in H

WEIGHT:

- STEMvision™: 59 lbs or 27 kg
- Computer: 28 lbs or 12 kg

POWER REQUIREMENTS:

- 100 - 240 V~, 50/60 Hz, 1.6 A
- Fuse 250V 2A Fast Blow

OPTIMAL OPERATING CONDITIONS:

- 15 – 30°C
- 20 – 85% relative humidity
- Not specified for use inside an incubator
- Does not require placement in a biohazard safety cabinet
- Indoor use only
- Not to be used in a cold room

STORAGE CONDITIONS:

- -30°C to 50°C
- 10 – 90% relative humidity

References

1. Migliaccio AR, et al. Blood 96: 2717-2722, 2000
2. Iori AP, et al. Bone Marrow Transplantation 33: 1097-1105, 2004
3. Yoo KH, et al. Bone Marrow Transplantation 39: 515 521, 2007
4. Prasad VK, et al. Blood 112: 2979-2989, 2008
5. Page KM, et al. Biol Blood Marrow Transplant 17: 1362-1374, 2011

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