Automated and Standardized Counting of CFU Assays of Human Hematopoietic Cells

STEMvision™

STEMcell™
TECHNOLOGIES
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## A Complete Set of Tools for the CFU Assay

STEMCELL Technologies, Inc. offers a comprehensive line of products for research laboratories, hematopoietic cell therapy laboratories and cord blood banks to determine the number of CFUs in cord blood, bone marrow and mobilized peripheral blood samples. STEMCELL Technologies’ Quality Management System is certified to ISO 13485 Medical Device Standards. Please visit us at [www.stemcell.com](http://www.stemcell.com) for additional information.
Standardized Counting of Colony-Forming Unit (CFU) Assays for Human Hematopoietic Cells

The colony-forming unit (CFU) assay is the gold standard in vitro functional assay for measuring the number of progenitor cells in human hematopoietic cell populations. The CFU assay has numerous applications for basic and clinical research in hematopoiesis, and in hematopoietic stem cell transplantation.

Historically, the CFU assay has been performed by culturing hematopoietic cells in MethoCult™ medium and counting the number of colonies produced by different sub-types of CFUs 14 days later using an inverted microscope. Colonies produced by different types of lineage-restricted and multi-potential progenitor cells (i.e. colony-forming unit-erythroid (CFU-E), burst-forming unit-erythroid (BFU-E), colony-forming unit-granulocyte, macrophage (CFU-G/M) and colony-forming unit-granulocyte, erythrocyte, macrophage and megakaryocyte (CFU-GEMM)), are identified and scored on the basis of well-defined morphological criteria. However, accurate counting of colony types can be challenging for individuals with limited experience. Manual counting of CFU assays is also time consuming and costly for laboratories that perform large numbers of assays each day.

STEMvision™ is a bench-top instrument and computer system designed specifically for automated imaging and counting of hematopoietic colonies in the CFU assay. This system has been optimized for use with MethoCult™ media and meniscus-free SmartDish™ cultureware (Figure 1). The use of this standardized platform significantly improves the accuracy and reproducibility of the human CFU assay.

Instead of manually identifying and counting colonies using a microscope, the user simply loads a SmartDish™ culture plate into STEMvision™ and the instrument performs these functions. STEMvision™ captures an image of each 35 mm well in approximately 1 minute and then uses highly sophisticated image analysis software to identify and classify each colony into the four major sub-types produced by CFU-E, BFU-E, CFU-G/M/GM and CFU-GEMM.

Users can choose to image and analyze each 35 mm well in a single step. Alternatively, for high-throughput processing, multiple dishes can be imaged sequentially and then analysed, in approximately 1 minute per well, overnight or at a later time, if desired.

Different Analysis Packages have been developed to accurately count CFUs in 14-day assays of human umbilical cord blood (CB), bone marrow (BM) and mobilized peripheral blood (MPB), using MethoCult™ Optimum. HetaSep™ has been developed to remove red blood cells (RBCs) from fresh samples prior to CFU analysis. RBC depletion improves the accuracy of the CFU assay whether colonies are counted manually or using STEMvision™. A faster CFU assay that uses MethoCult™ Express has also been developed specifically for CB banks, enabling the number of CFUs in CB units to be measured in only 7 days. This can allow results to be obtained in sufficient time to inform decision-making around CB transplantation.

A Complete Workflow For Human Hematopoietic CFU Assays:
- Primary human hematopoietic cells
- EasySep™ and RoboSep™ for isolation of hematopoietic stem and progenitor cells
- HetaSep™ for removing red blood cells from fresh samples
- MethoCult™ Express and MethoCult™ Optimum media for 7- and 14-day CFU assays, respectively
- SmartDish™ meniscus-free cultureware for more reliable colony counting
- STEMvision™ for automated and standardized colony counting
- Analysis Packages for counting the four major CFU subtypes in human CB, BM and MPB
- Proficiency testing programs
The CFU Assay Workflow

Figure 1. A Typical CFU Assay Workflow Incorporating STEMvision™ for Automated Counting of Hematopoietic Colonies

Red blood cells (RBCs) are removed from 50 μL of fresh cord blood (CB), bone marrow (BM) or mobilized peripheral blood (MPB) samples using HetaSep™. This step is not required for thawed cryopreserved cells. Fresh or thawed cells are then cultured in SmartDish™ containing the appropriate MethoCult™ medium, depending on the cell type and whether the CFU assay will be counted after 7 or 14 days. STEMvision™ acquires an image of each culture, and then classifies and counts the number of colonies produced by the four major subtypes of hematopoietic progenitor cells; CFU-E, BFU-E, CFU-G/M/GM and CFU-GEMM. STEMvision™ can generate a printed report of the CFU assay results documenting the frequency and total number of CFUs in the sample. For cord blood banks, an additional report can be produced for the family if desired.
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 human colony-forming unit (CFU) assays. 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). Several Analysis Packages have been developed to score CFUs in 14-day assays of cord blood (CB), bone marrow (BM) and mobilized peripheral blood (MPB) cells. The progenitor cells in these tissues exhibit subtle differences in colony morphology that are challenging to distinguish without extensive training and experience.

By using an automated system to standardize colony counting, research labs, transplant labs and CB banks can ensure that their CFU assay results are accurate and reproducible.

Figure 2. Representative STEMvision™ Images Showing Colonies Derived from CB Progenitors After 7 Days of Culture in MethoCult™ Express, and From CB, BM and MPB After 14 Days of Culture in MethoCult™ Optimum

These images have been analyzed by STEMvision™ Human (A) 7-Day and (B-D) 14-Day Analysis Packages. Green circles identify individual colonies in a 7-day CB CFU assay that scores total CFUs only (A). Orange and red circles identify erythroid colonies (produced by CFU-E and BFU-E, respectively), 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 14-day CB (B), BM (C) and MPB (D) CFU assays. Erythroid and mixed colonies that contain hemoglobinized cells are shown in true red color.
**STEMvision™ Performance Data**

Automated 14-Day CFU Assays of Human Cord Blood Cells

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

Cryopreserved CB cells were thawed, plated in MethoCult™ Optimum and cultured for 14 days. The resulting colonies were then counted both manually using an inverted microscope, and automatically using STEMvision™. 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

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**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 significantly 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.
Automated 14-Day CFU Assays of Human Bone Marrow Cells

Figure 5. STEMvision™ Automated Scoring of Total, Erythroid (BFU-E + CFU-E) and Myeloid (CFU-G/M/GM) Colonies is Highly Correlated to Manual Counts of 14-Day BM CFU Assays

Cryopreserved BM cells were thawed, plated in MethoCult™ Optimum, cultured for 14 days, and the resulting colonies then scored both manually using an inverted microscope and automatically using STEMvision™. The BM Analysis Package can identify and count erythroid colonies produced by BFU-E and CFU-E separately, but these are combined in panel B. 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=120 CFU assays) are as follows:

- Figure 5A: y=0.88x + 8.79; R^2=0.95 for Total Colonies
- Figure 5B: y=0.83x + 6.71; R^2=0.89 for BFU-E + CFU-E
- Figure 5C: y=0.92x + 2.55; R^2=0.94 for CFU-G/M/GM

Figure 6. STEMvision™ Automated Scoring of Mixed Colonies Falls Within the Range of Manual Counts of 14-Day BM CFU Assays

Thirty individual 14-day BM CFU assays were counted by three to seven people. The numbers of mixed (CFU-GEMM) colonies counted manually in each well is shown as open circles (n=82 total assay scores). Manual CFU-GEMM counts in most cultures varied significantly 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.
Automated 14-Day CFU Assays of Human Mobilized Peripheral Blood Cells

Figure 7. STEMvision™ Automated Counting of Total, Erythroid (BFU-E) and Myeloid (CFU-G/M/GM) Colonies is Highly Correlated to Manual Counts of 14-Day MPB CFU Assays

Cryopreserved MPB cells were thawed, plated in MethoCult™ Optimum, cultured for 14 days, and the resulting colonies then scored both manually using an inverted microscope and automatically using STEMvision™. 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 (R²) that describe each data set (n=143 CFU assays) are as follows:

Figure 7A: y=0.97x + 2.44; R²=0.97 for Total Colonies
Figure 7B: y=0.96x + 3.74; R²=0.91 for BFU-E
Figure 7C: y=0.96x + 0.90; R²=0.95 for CFU-G/M/GM

Figure 8. STEMvision™ Automated Scoring of Mixed Colonies Falls Within the Range of Manual Counts of 14-Day MPB CFU Assays

Thirty individual 14-day MPB 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=82 total assay scores). Manual CFU-GEMM counts in most cultures varied significantly 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.

FOR RESEARCH USE ONLY. NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES.
STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485 MEDICAL DEVICE STANDARDS.
Another important advantage of STEMvision™ for automated and standardized counting of hematopoietic colonies in human CFU assays is significantly improved reproducibility of assay results. The coefficient of variation in STEMvision™ colony counts is two- to three-fold lower in the recommended range of 20 - 80 colonies per 35 mm culture well than for counts produced by multiple technicians who manually count the same CFU assays.

The reduced variability of automated colony counts in 7-day and 14-day CFU assays of CB cells is shown in Figure 9 (A) and (B) respectively.

Figure 9. STEMvision™ Automated Colony Counting of 7-Day and 14-Day CB CFU Assays is More Reproducible Than Manual Counting

The coefficients of variation (CVs) for total colony counts in (A) 7-day and (B) 14-day CFU assays of CB cells were determined by counting the same culture dishes 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%.
Figure 10. Sample STEMvision™ Lab Report for a 14-Day Cord Blood CFU Assay

STEMvision™ produces two printed reports that detail information about the specific hematopoietic cell sample and the colony-forming unit (CFU) assay results (Figure 10). These reports provide critical functional information about the cell sample for the research or clinical laboratory’s own records. In the case of cord blood (CB) banking, a second Parent Report form (not shown) can be produced for parents banking their child’s CB if desired. The user-customizable information documented in these reports include:

- Laboratory address and contact information
- Patient and doctor demographic information
- Total number of viable progenitors in sample
- Sample and CFU assay tracking ID numbers
- CFU counts expressed per 100,000 nucleated cells or per mL of sample
- Assay counts for CFU-E, 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).
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 sample

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™ (Figure 11). RBCs must therefore be removed from fresh cord blood, bone marrow and mobilized peripheral blood 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 sample 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 11. 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.
Automated and Standardized Colony Counting

**MethoCult™ Express & MethoCult™ Optimum**

Methylcellulose Media for 7-Day and 14-Day CFU Assays

STEMvision™ has been designed for use with MethoCult™ media in order to ensure optimal colony growth, counting and CFU assay accuracy. The 14-Day Analysis Packages (Catalog #22005, #22006, #22007) are used with MethoCult™ Optimum (Catalog #04034/04044) medium which supports optimal growth of erythroid progenitors (CFU-E and BFU-E), granulocyte/macrophage progenitors (CFU-G, CFU-M and CFU-GM) and multi-potential granulocyte, erythrocyte, macrophage, megakaryocyte progenitors (CFU-GEMM) from human cord blood (CB), bone marrow (BM) and mobilized peripheral blood (MPB).

The Human Cord Blood 7-Day Analysis Package (Catalog #22001) is used with MethoCult™ Express (Catalog #04437/04447) medium. MethoCult™ Express is formulated to accelerate the proliferation of human hematopoietic progenitor cells in CB and thus allows colonies to be counted after only 7 days; one week faster than with a conventional 14-day CFU assay. 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. The 7-day CFU assay provides a simple method to determine the total number of viable and functional progenitor cells in a CB unit, without discriminating between the different sub-types of CFUs. Several clinical studies have shown that the total number of CFUs in a CB unit is the single parameter that correlates most strongly with engraftment outcomes following CB transplantation.1-5


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

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

<table>
<thead>
<tr>
<th>METHOCULT™ PRODUCT</th>
<th>CATALOG #</th>
<th>SIZE</th>
<th>COMPONENTS</th>
<th>APPLICATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MethoCult™ Express</td>
<td>04437*</td>
<td>100 mL 24 x 3 mL</td>
<td>Cytokines, including erythropoietin (EPO)</td>
<td>Measures the total number of CFUs in human CB in only 7 days</td>
</tr>
<tr>
<td></td>
<td>04447*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MethoCult™ Optimum</td>
<td>04034/84434*</td>
<td>100 mL 24 x 3 mL</td>
<td>Cytokines, including erythropoietin (EPO)</td>
<td>Supports growth of CFU-E, BFU-E, CFU-G/M/GM and CFU-GEMM in human CB, BM and MPB</td>
</tr>
<tr>
<td></td>
<td>04044/84444*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MethoCult™ Optimum without EPO</td>
<td>04035/84534*</td>
<td>100 mL 24 x 3 mL</td>
<td>Cytokines, with no rhEPO</td>
<td>Supports growth of CFU-G, CFU-M and CFU-GM in human CB, BM and MPB</td>
</tr>
<tr>
<td></td>
<td>04045/84544*</td>
<td></td>
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</tr>
</tbody>
</table>

MC: methylcellulose; FBS: fetal bovine serum; BSA: bovine serum albumin; CB: cord blood; BM: bone marrow; MPB: mobilized peripheral blood

*CE Marked for IVD use in the EU.

**Please contact Tech Support for more information.**
**SmartDish™**

**Meniscus-Free Cultureware for More Accurate Counting of Hematopoietic Colonies**

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 13A), 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 13B). 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 13. 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).
Automated and Standardized Colony Counting

**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

**PRODUCT:** STEMvision™ Human Bone Marrow  
14-Day CFU Analysis Package  
**CATALOG #:** 22006

**PRODUCT:** STEMvision™ Human Mobilized Peripheral Blood  
14-Day CFU Analysis Package  
**CATALOG #:** 22007

**SYSTEM IS SUPPLIED WITH:**
- STEMvision™ base unit (#22102C)  
- Computer and monitor (#22101)  
- Software for image acquisition, analysis and review  
(Catalog #22001, #22005, #22006 or #22007 as selected)  
- One- or two-year warranty

**REQUIRED REAGENTS:**
- HetaSep™ (page 12)  
- MethoCult™ Express or MethoCult™ Optimum (page 13)  
- SmartDish™ cultureware (page 14)

**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/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**

2. Iori AP, et al., Bone Marrow Transplantation 33: 1097-1105, 2004

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