Proficiency Testing
For Hematopoietic Progenitor Assays Using Bone Marrow

For Reducing Variability and Standardization of Human Hematopoietic Colony Assays

Introduction

STEMCELL Technologies Inc. is committed to standardizing hematopoietic colony assays. Despite the advent of standardized media (e.g. MethoCult™), significant variability in assay results performed by individuals still hinders the ability to directly compare data generated at different centers. This variability has been associated with both sample preparation[1] and differences in colony identification and enumeration.[2] In order to address the issues arising from the variability encountered in these two areas, STEMCELL Technologies designed a two-step proficiency testing approach. Stage One of the program ran bi-annually from 1998 to 2000 in selected countries and focused exclusively on participants' proficiency at colony identification and enumeration. The aim of reducing the variation in colony scoring among participants was achieved: the coefficient of variation observed during the course of the program[3] was significantly lower than previously published reports.[2,4]

STEMCELL Technologies' Proficiency Testing Program has now advanced to Stage Two. In addition to colony identification and enumeration, this program incorporates upstream events, including cell thawing, dilution, inoculation and plating. Like STEMCELL Technologies' first Proficiency Testing Program, this program runs bi-annually and maintains the anonymity of all participants. Unlike the initial program, however, there is flexibility in the timing of assay initiation, and participation is available worldwide. This proficiency testing program continues to reduce the variability associated with colony assays and helps individuals to identify specific areas that might benefit from continuing education and training.

Background

Since their introduction more than 30 years ago, colony assays have been used extensively for research and clinical applications. These include identification of stimulatory and inhibitory growth factors, supportive diagnostic assays of myeloproliferative disorders and leukemias, and evaluation of the hematopoietic proliferative potential of bone marrow, cord blood and mobilized peripheral blood samples for clinical transplantation.[5] Studies at some clinical institutions have found a correlation between progenitor content, as assessed by colony assay, and engraftment[6,7] whereas others have found no such associations.[8,9]

CD34 antigen expression is often used as a surrogate marker for hematopoietic stem cells and enumeration of $CD34^+$ cells has been used to quantify progenitor and stem cell content.[10] This assay, although informative, does not evaluate the functional capacity of the cell subsets which support hematopoiesis in transplant recipients. The colony assay, on the other hand, is the benchmark functional assay to assess the ability of hematopoietic cells from various sources to divide and differentiate, especially following ex vivo manipulations including T cell depletion, $CD34^+$ cell enrichment, gene therapy protocols and cryopreservation. Therefore, performing functional tests for progenitors provides important complementary information to $CD34^+$ cell enumeration.

Description of Proficiency Test

Participants will receive a worksheet providing instructions for each step in the proficiency test, as well as a Technical Manual (for first time participants) containing detailed protocols for CFU assays using MethoCult™. Participants will be assessed on their proficiency at performing colony assays for bone marrow samples, focusing on the following aspects:

1. Cell Counting, Preparing Cell Dilution and Cell Inoculation

Participants will be provided with frozen bone marrow cells*, MethoCult™ methylcellulose-based medium containing recombinant cytokines, and additional reagents and supplies required for initiating cultures.

Participants will thaw frozen BM cells, perform a TNC count, assess sample viability and prepare an appropriate pre-determined cell dilution to inoculate in MethoCult™ medium for the CFU assay. Cultures will be incubated for 14 days at 37°C, 5% CO_2.

*Note: Human cells have been screened and found to be negative for Hepatitis B, C and HIV-1, however, they should be considered potentially infectious and handled accordingly.

2. Colony Enumeration

Participants will be provided with a gridded scoring dish to assist with colony enumeration.

Following 14 days in culture, participants will enumerate the following: CFU-E, BFU-E, CFU-GM and CFU-GEMM.

3. Colony Identification

Photographs of CFUs from human bone marrow samples will be posted on our website for identification purposes.

Participants will be tested for their ability to identify granulocyte/macrophage, erythroid and multi-lineage colonies from the photographs displayed.

Submission of Results

Prior to a specified deadline, participants will submit data to STEMCELL Technologies for compilation and statistical analysis. The worksheet provided can be used for direct entry of assay results. Data can be submitted electronically via our website (www.proficiencytesting.com). Alternatively, completed forms can be submitted to STEMCELL Technologies by fax transmission or e-mailed to proficiency@stemcell.com.

Analysis of Data

STEMCELL Technologies will compile and statistically analyze all valid data received by the submission deadline. A comprehensive report including graphs and tables will be generated and returned to each participant. Participants will be able to determine their individual data points via anonymous ID numbers.

For additional information, please visit our website at www.proficiencytesting.com or www.stemcell.com, or contact us at our dedicated e-mail address: proficiency@stemcell.com.

References

1. Burger SR, Kadidlo D, McCullough J: Transfusion 39: 451, 1999
2. Lumley MA, Burgess R, Billingham LJ, et al: Br J Haematol 97: 481, 1997
3. Clarke E, Mantie D, Miller CL et al: Blood 94 (10):140a, 1999 (abstract)
4. Keever-Taylor CA, Collins NH, Carter S, et al: Blood 90 (10): 368a, 1997 (abstract)
5. Gordon MY: Blood Rev 7: 190, 1993
6. Bacigalupo A, Piaggio G, Podesta M, et al: Bone Marrow Transplant 15: 221, 1995
7. Cancelas JA, Querol S, Canals C, et al: Transfusion 38: 1063, 1998
8. Torres A, Alonso MC, Gomez-Villagran JL, et al: Blut 50: 89, 1985
9. Masszi T, Gluckman E: Acta Biomed Atenco Parmense 64: 221, 1993
10. Sutherland R, Stewart AK, Keating A: Stem Cells 11 (suppl 3): 50, 1993

Bone Marrow Proficiency Testing Program

Registration Form for Countries Serviced by a Distributor

If you have any questions, please contact us at: proficiency@stemcell.com. Space is limited, please register early.

June 2015

For registration in our June 2015 Bone Marrow Proficiency Testing Program, completed forms must be received no later than **April 30, 2015**.

PARTICIPANT FIRST AND LAST NAME	E-MAIL ADDRESS
1.	
2.	
3.	
4.	
5.	

October 2015

For registration in our October 2015 Bone Marrow Proficiency Testing Program, completed forms must be received no later than **September 18, 2015**.

PARTICIPANT FIRST AND LAST NAME	E-MAIL ADDRESS
1.	
2.	
3.	
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5.	

An e-mail address is required for each participant. All correspondence with participants, including cell plating concentration and final reports, is done via e-mail.

Customer Information

Institution: _____

Shipping Address: _____

Department: _____

Principal Investigator: _____ Email: _____

Phone Number: _____ Fax Number: _____

Shipping Address: _____

Signature: _____

Program Costs

Please contact your regional distributor to register the first participant from a laboratory (Catalog #00602) or additional participants from a laboratory (Catalog #00603). Our distributor's contact information are listed at the following link, please select your country from the drop-down menu: **www.stemcell.com/en/Contact-Us.com**.

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FOR FULL CONTACT DETAILS WORLDWIDE VISIT OUR WEBSITE