Cytotherapy, 2013; 15: 255–262

International Society for Cellular Therapy
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ORIGINAL PAPERS

Current practices and prospects for standardization of the hematopoietic colony-forming unit assay: a report by the cellular therapy team of the Biomedical Excellence for Safer Transfusion (BEST) Collaborative

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Abstract

Background aims. Wide acceptance of the colony-forming unit (CFU) assay as a reliable potency test for stem cell products is hindered by poor inter-laboratory reproducibility. The goal of this study was to ascertain current laboratory practices for performing the CFU assay with an eye towards identifying practices that could be standardized to improve overall reproducibility. *Methods.* A survey to evaluate current laboratory practices for performing CFU assays was designed and internationally distributed. *Results.* There were 105 respondents to the survey, of whom 68% performed CFU assays. Most survey recipients specified that an automated rather than a manual cell count was performed on pre-diluted aliquots of stem cell products. Viability testing methods employed various stains, and when multiple sites used the same viability stain, the methods differed. Cell phenotype used to prepare working cell suspensions for inoculating the CFU assay differed among sites. Most respondents scored CFU assays at 14–16 days of incubation, but culture plates were read with various microscopes. Of 57 respondents, 42% had not performed a validation study or established assay linearity. Only 63% of laboratories had criteria for determining if a plate was overgrown with colonies. *Conclusions.* Survey results revealed inconsistent inter-laboratory practices for performing the CFU assay. The relatively low number of centers with validated CFU assays raises concerns about assay accuracy and emphasizes a need to establish central standards. The survey results shed light on numerous steps of the methodology that could be targeted for standardization across laboratories.

Key Words: *colony-forming-units, hematopoietic, hematopoietic progenitor cells, potency test*

Introduction

Functional analysis of hematopoietic progenitor cell (HPC) products is critical for comparative selection of the highest quality stem cell product for a transplant recipient. However, the selection of stem cell products for transplantation is typically based primarily on non-functional cellular parameters such as total nucleated cell (TNC) counts and cellular immunophenotypes (e.g., $CD34^+$ cell counts). Although these surrogate assays have demonstrated good inverse correlations

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(Received 9 July 2012; accepted 11 November 2012)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2012.11.013

with the period of clinically relevant cytopenia, the predictive value of these assays for hematopoietic engraftment may be reduced because they do not provide functional information on the hematopoietic quality of the graft. The absence of functional hematopoietic information may result in the selection of low-potency stem cell products that fail to engraft in a patient despite a unit having a high cell count with an acceptable phenotype.

The colony-forming unit (CFU) assay is a hematopoietic functional assay that is often used to measure the function or potency of hematopoietic progenitors present in stem cell products. However, poor inter-laboratory reproducibility of the CFU assay even among experienced laboratories precludes universal implementation of this assay (1,2). As a consequence, the CFU assay fails to meet potency testing guidelines as set forth by the U.S. Food and Drug Administration (3). These guidelines require that a potency assay be capable of predicting therapeutic outcome, establishing industry release criteria and defining product expiration.

Reasonably good intra-laboratory reproducibility for the CFU assay has resulted in some investigators reporting that there is a good correlation between numbers of CFU-generating progenitors present in stem cell products and short-term hematopoietic reconstitution in autologous and allogeneic transplantation settings (4–9). Given that CFU assays performed at a single site can correlate with engraftment, it should be possible with stringent standardization of the method to improve inter-laboratory reproducibility so that results from different sites can be used to predict the *in vivo* efficacy of stem cell grafts for clinical applications.

The CFU assay takes advantage of the ability of a HPC to proliferate and differentiate to form a colony of cells committed to specific blood cell lineages. This *in vitro* assay is typically performed by removing an aliquot of cells from a stem cell product, preparing a working cell suspension, inoculating growth factor-containing semi-solid medium with a desired cell concentration and transferring cells and methylcellulose into culture dishes (Figure 1). The dishes are placed in a humidified incubator for a defined period. At the end of the culture period, the total number of colonies produced is counted microscopically and classified according to their morphologic features as burst-forming unit-erythroid (BFU-E), colony-forming unit erythroid (CFU-E), colonies containing granulocytes and macrophages (CFU-GM), and colonies containing granulocytes, erythrocytes, macrophages and megakaryocytes (CFU-GEMM). The type and number of the colonies obtained at the end of the culture period are driven by the amount and combination of growth factors present in the culture.

As a first step toward inter-laboratory standardization of the CFU assay, the cellular therapy team of the Biomedical Excellence for Safer Transfusion (BEST) Collaborative designed a survey to evaluate current practices among different laboratories to identify sources of variability that may contribute to assay variability. The survey focused on practices associated with performing the CFU assay on fresh samples and was distributed internationally through membership rosters of the American Association of Blood Banks (AABB), International Society for Cell Therapy (ISCT) and European Group for Blood and Marrow Transplantation (EBMT) societies. Results from the survey expose highly variable laboratory practices, which support a need for the establishment of inter-laboratory standards for the CFU assay. Using survey results, we provide in this article suggestions for areas of practices to be considered for standardization

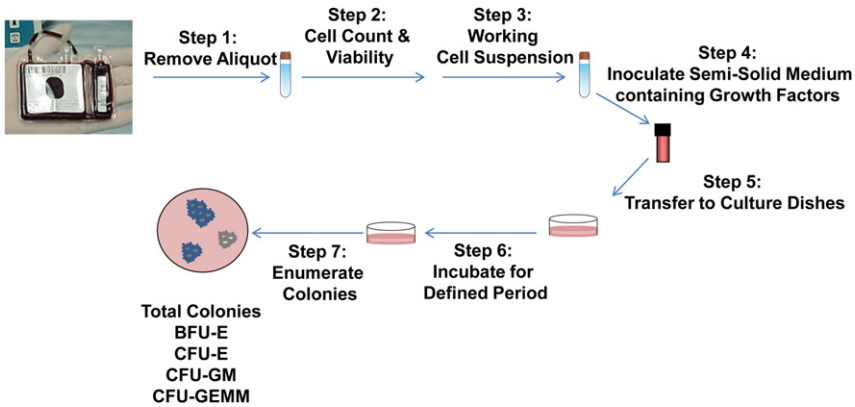

Figure 1. Basic steps for setting up the CFU assay. Step 1: An aliquot of cells is removed from a stem cell product. Step 2: A pre-dilution cell count and viability test are performed. Step 3: A working cell suspension is made from the aliquot of cells removed from step 1. Step 4: Semi-solid medium containing growth factors is inoculated with a defined volume of the working cell suspension. Step 5: The semi-solid medium containing growth factors and cells is transferred to a culture vessel. Step 6: The culture vessel is placed in an incubator for a defined period. Step 7: At the end of the culture period, the colonies are enumerated and differentiated.