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A novel user-independent CFU assay for hematopoietic stem and progenitor cells

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Introduction

The current gold standard for the analysis of hematopoietic stem and progenitor cells (HSPCs) is the colony-forming–unit (CFU) assay. Cells are plated in 35 mm dishes or 6-well plates with a methylcellulose-based medium and incubated for 14 days. This leads to different numbers and types of colonies, which can be analyzed visually, one by one, with a microscope. Based on these results, the proliferation and differentiation potential of the initial sample can be evaluated. This analytical method, however, is highly dependent on the judgement and experience of each individual researcher counting the colonies.

The StemMACS™ HSC-CFU Assay Kit was developed to eliminate the user-dependent bias, while maintaining the performance of the assay. The kit consists of a newly developed medium for the cultivation of the cells in 96-well plates, as well as a specifically designed antibody cocktail, which allows the identification of each colony type based on their surface markers by flow cytometry.

Results

1 Experimental setup and workflow of the StemMACS™ HSC-CFU Assay Kit

In contrast to the standard CFU assay, which uses larger cell culture dishes, the cells were plated in 96-well plates and analyzed by flow cytometry (fig. 1).

CD34⁺ HSPCs were diluted in Iscove´s Modified Dulbecco Media (IMDM) to a density of 2.5 cells/10 µL and seeded in each well of three 96-well plates, followed by addition of 50 µL of the StemMACS™ HSC-CFU Assay Medium (fig. 2A). The goal of the dilution step was to have one colony-forming cell per well. Subsequently, the plates were placed in a humidified incubator at 37 °C for 14 days, during which the CD34⁺ cells proliferated and differentiated (fig. 2B). After the incubation period, the cells were stained with the StemMACS HSC-CFU Assay Cocktail (fig. 2C). Subsequently, PBS/EDTA buffer with 0.5% BSA (PEB) was added to obtain a total volume of 100 µL per well (fig. 2D). Finally, the samples were analyzed by flow cytometry on a MACSQuant® Analyzer.

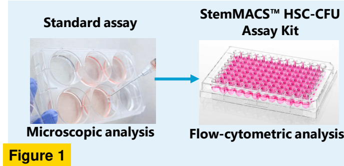

Figure 1

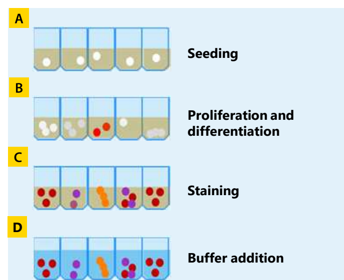

Figure 2

2 Flow cytometric analysis

After acquisition of the samples the results were visualized with an appropriate software and analyzed according to the following gating strategy: First, all cells acquired were selected (fig. 3A) and out of those the single cells were gated (fig. 3B). From the single cells two new dot plots were created, one showing the CD15-APC vs. CD235a-PE staining (fig. 3C), and the other displaying the CD15-APC vs. CD14-VioBlue® staining (fig. 3D).

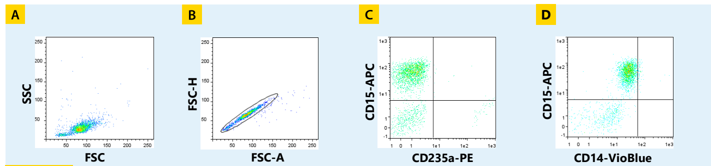

Figure 3

Subsequently, the flow cytometric data were exported to a spreadsheet in order to analyze the entire plate. Based on whether the cells exhibited a single positive signal in one of the fluorescence channels, or positive signals in two or three channels, it was possible to determine which colony type was present in each well (table 2). For example, the cells of a CFU-GEMM colony were CD235a-, CD15-, and CD14 positive (table 2 and fig. 4E). All other colony types were identified in a similar way (fig. 4).

Specificity	Fluorochrome	AB clone	Cell type
CD235a	PE	REA175	Erythrocytes
CD15	APC	VIMC6	Granulocytes
CD14	VioBlue	TÜK4	Monocytes

Table 1

Colony	Positive staining for
BFU-E	CD235a-PE
CFU-G	CD15-APC
CFU-M	CD14-VioBlue
CFU-GM	CD15-APC & CD14-VioBlue
CFU-GEMM	CD235a-PE & CD15-APC & CD14-VioBlue

Table 2

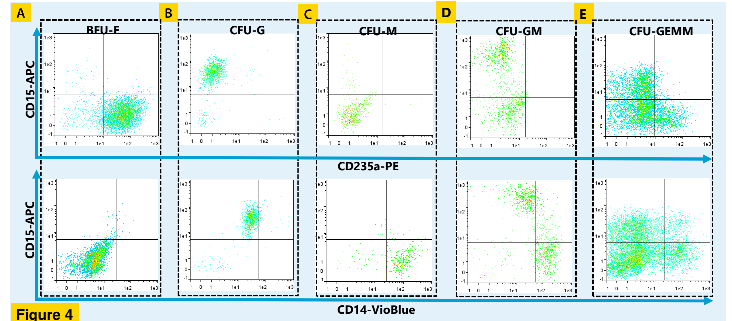

Figure 4

3 Colony counts and types are highly comparable between the standard and flow cytometry-based CFU assay

We compared the results obtained with the standard 6-well–based assay to the ones from the StemMACS HSC-CFU Assay Kit in order to assess the reproducibility and comparability of the new format. CD34⁺ cells from different sources (mobilized leukapheresis, cord blood, peripheral blood mononuclear cells [PBMCs]) were prepared and seeded in the respective media for the standard assay (6-well plates; two wells per donor; 250 HSPCs/well) or the StemMACS HSC-CFU Assay Kit (96-well plates; three plates per donor; 250 HSPCs/plate). The plates were incubated for 14 days at 37 °C, 5% CO_2. Afterwards, the cells were analyzed with a microscope (standard assay) or with the MACSQuant Analyzer (StemMACS HSC-CFU Assay Kit). The numbers for both total colony counts (fig. 5A) and individual colony types (fig. 5B) were highly comparable between the two methods. The results were reproducible for all materials used and across multiple donors.

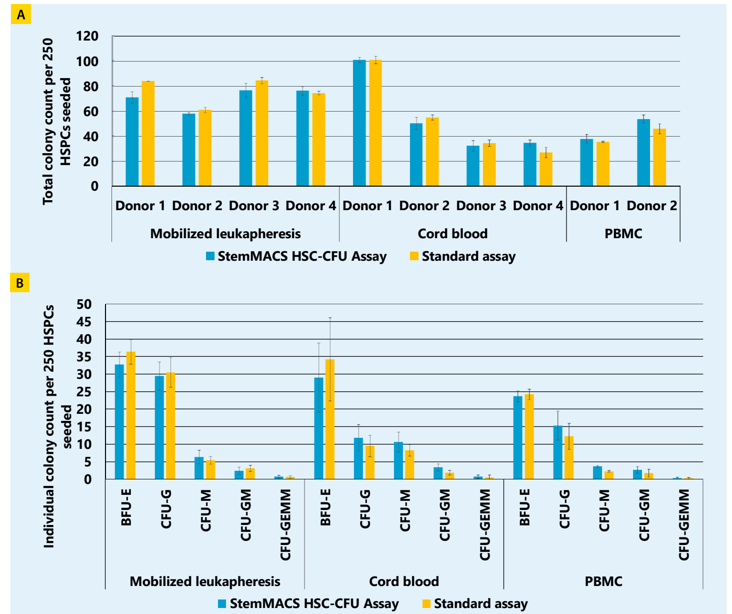

Figure 5

Conclusion

- The StemMACS HSC-CFU Assay Kit offers a highly standardized method for analyzing HSPCs.
- The flow cytometry–based readout eliminates the need for user-dependent, visual scoring under a microscope.
- Flow cytometry analysis is amenable to automation and thus allows for high throughput .
- Results were very similar between the standard CFU assay and the newly developed StemMACS HSC-CFU Assay Kit.

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