Performance of commercial methylcellulose media for short-term colony assays in routine transplantation practice

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Introduction

CD34⁺ cell counts are required to guide apheresis but a functional assay for granulocyte monocyte-colony forming cells, GM-CFC (CFU-GM) is essential where cells may be damaged by cryopreservation, storage or processing. Furthermore, where patients for autograft transplantation achieve a marginal CD34⁺ cell dose of 1–2×10⁶/kg it is our practice to use the GM-CFC dose as a guide to progenitor quality and proceed if this is at least 20×10⁴/kg. Our routine methylcellulose assay media for many years was Stem Cell Technologies "base media" H4230 with our own added cytokines ("UCL-mix", table 1). We have evaluated five commercially available medias stated to be suitable for routine GM-CFC and BFUE counts. Four were from Stem Cell Technologies (SCT) and one from Miltenyi Biotec (MACS Media). Two preparations showed relatively poor GM-CFC growth that appeared to relate to lower growth factor concentrations. In addition to laboratory comparisons, the relationship between CD34⁺ cell and GM-CFC doses of apheresis harvests was compared against historical data for two of the media selected as most suitable for routine use.

Methods and results

Samples tested in parallel in methylcellulose media

A consecutive series of routine stem cell samples were plated in parallel to compare methylcellulose media formulations. The resulting number of GM-CFC or BFU-E colonies counted per well were compared and significant differences assessed using paired t-tests (fig. 1a–e). A total of 148 samples were tested in parallel. 129 were routine HPC,A harvests, 3 were bone marrow harvests, 8 were purified CD34⁺ cells, and 8 were depleted CD34⁺ cell samples from clinical CD34 cell selection procedures. Apheresis and bone marrow MNC samples were plated in media at 2.5×10⁴/mL and purified CD34⁺ or depleted CD34⁺ cell samples were plated at 5×10³/mL and 5×10⁵/mL, respectively. Apheresis harvests with a CD34⁺ cell concentration >3% were plated at 2.5×10³/mL. The cells in media were plated in quadruplicate in 24-well Costar culture plates and counted after 14 days incubation in a well-humidified incubator at 37 °C and 5% $C0_2$ concentration. H4434 was evaluated initially but was found to be suboptimal for GM-CFC growth against the UCL media (fig. 1a). H4434 does not include G-CSF and supplementing H4434 with 20 ng/mL G-CSF fully restored GM-CFC growth (data not shown). H4435 however was comparable with the in-house UCL media (fig. 1b) and was introduced into routine use in August 2006.

Figure 1a–e: Paired stem cell harvest comparison of colony growth in different methyl cellulose preparations

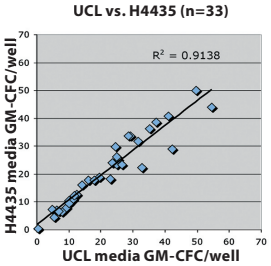


Figure 1a: UCL vs. H4434 media - CFC/well

	GM-CFC/well		BFU-E/well	
	UCL	H4434	UCL	H4434
Mean	18.1	11.1	24.8	27.3
1 SD	15.1	9.8	18.5	20.0
T-test	<0.001		NS	

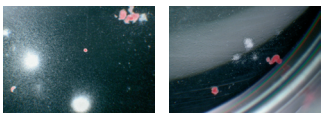


Figure 2: Typical GM-CFC appearances in UCL media (left) and H4434 (right)

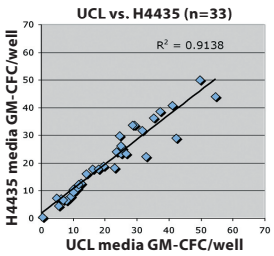


Figure 1b: UCL vs. H4435 media - CFC/well

	GM-CFC/well		BFUE/well	
	UCL	H4435	UCL	H4435
Mean	21.8	21.1	31.5	29.6
1 SD	13.7	12.7	20.0	17.5
T-test	NS		NS	

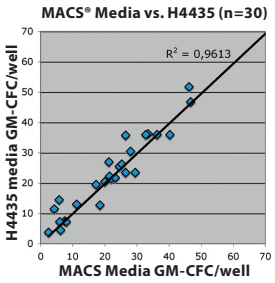


Figure 1c: MACS Media vs. H4435 media - CFC/well

	GM-CFC/wel		BFU-E/well	
	MACS	H4435	MACS	H4435
Mean	26.8	27.7	30.7	31.7
1 SD	23.4	22.2	21.8	22.5
T-test	NS		NS	

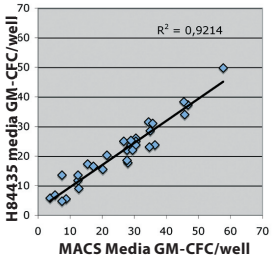


Figure 1d: MACS Media vs. H84434 media - CFC/well

	GM-CFC/well		BFU-E/well	
	MACS	H84434	MACS	H84434
Mean	26.0	21.5	22.4	19.9
1 SD	10.5	10.5	12.8	11.4
T-test	<0.001		<0.001	

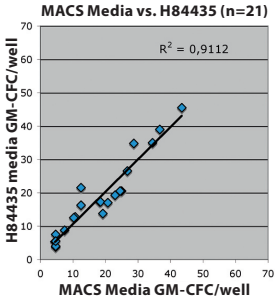


Figure 1e: MACS Media vs. H84435 media - CFC/well

	GM-CFC/well		BFU-E/well	
	MACS	H84435	MACS	H84435
Mean	18.0	21.5	20.1	19.3
1 SD	11.5	11.6	13.8	12.8
T-test	NS		NS	

Table 1: Growth factor concentrations in the methylcellulose media preparations tested

Factor	Units	UCL	H4434	H4435	MACS	H84434	H84435
SCF	ng/mL	10	50	50	50	50	50
IL-3	ng/mL	30	10	10	20	10	20
GM-CSF	ng/mL	25	10	10	20	10	20
G-CSF	ng/mL	25	–	20	20	10	20
IL-6	ng/mL	–	–	20	20	–	–
EPO	U/mL	2	3	3	3	3	3

The basic composition of all media was the same, comprising 1% methylcellulose in Iscove's MDM, 30% fetal bovine serum, 1% bovine serum albumin, 2-mercaptoethanol and 2 mM L-glutamine

MACS Media 130-091-280 was subsequently compared to H4435 (fig. 1c) and was also deemed suitable for routine use. Two formulations of CE-certified Methocult were recently introduced by Stem Cell Technologies for the European market (H84434 and H84435, table 1). These were compared with MACS Media 130-091-280 which was in routine clinical use since May 2007. GM-CFC and BFU-E counts were systematically around 20% lower with H84434 probably related to lower cytokine concentrations compared to the MACS Media (fig. 1d, table 1). In contrast H84435 and MACS Media colony assays compared very well both in size and number of GM-CFC and BFU-E colonies (fig. 1e). All of the media on test supported BFU-E colony growth. Although not used for clinical purposes, BFU-E were included as an internal indicator of good culture conditions.

Harvest CD34: GM-CFC ratios

We have previously shown a close (though non-linear) correlation between apheresis harvest CD34$^+$ cell and GM-CFC doses and the "clonogenicity" of CD34$^+$ cells (CD34:GM-CFC) can be expressed as a ratio. Thus a harvest with a CD34 dose of 4×10^6/kg and GM-CFC of 80×10^4/kg has a CD34:GM-CFC ratio of 0.2. Mean harvest CD34:GM-CFC ratios were compared where UCL, H4435, or MACS Media had been used for GM-CFC assays (table 2) and showed similar patterns to historical data (fig. 3) for consecutive series of routine autologous or allogeneic apheresis harvests.

Table 2. Mean (SD) CD34:GM-CFC ratio of autologous and allogeneic apheresis harvests using different methylcellulose media for GM-CFC assays

CD34:GM-CFC		
UCL media	**ratio**	**SD**
HPC,A (auto =274)	0.22	0.09
HPC.A (allo = 177)	0.26	0.08
H4435 media		
HPC,A (auto =104)	0.22	0.08
HPC.A (allo = 177)	0.28	0.11
MACS Media		
HPC,A (auto = 74)	0.23	0.10
HPC.A (allo = 91)	0.28	0.08

Figure 3: Distribution of CD34:GM-CFC ratios of HPC,A (auto) harvests (n=274) using UCL media for GM-CFC assays

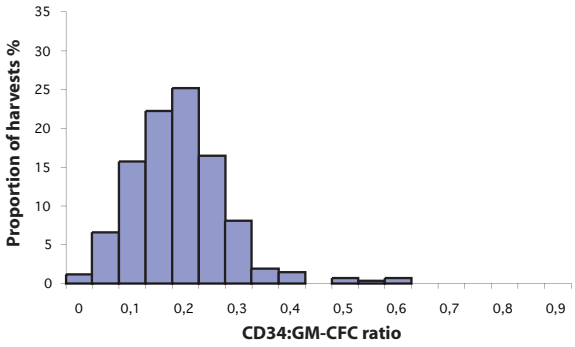
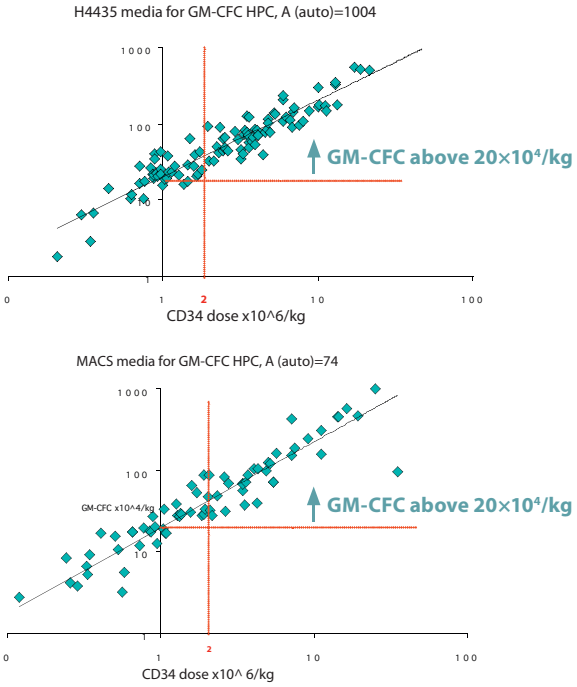


Figure 4ab: An HPC,A (auto) CD34$^+$ cell dose of 2×10^6/kg assures a GM-CFC dose of 20×10^4/kg



Progenitor collection thresholds for HPC,A (auto)

We have previously observed that an apheresis collection target of 2×10^6/kg CD34$^+$ cells for autologous harvests ensures a GM-CFC dose of 20×10^4/kg in almost all cases. This was the case for all harvests assessed whether H4435 or MACS Media was used for GM-CFC assay (fig. 4ab).

Conclusions

Maximal GM-CFC growth appeared to be highly dependent on the methylcellulose cytokine composition and was poor when G-CSF was omitted or was below 20 ng/mL.

Stem Cell Technologies H4435 and MACS Media 130-091-280 performed equally well both in laboratory testing and in routine clinical practice compared to previous experience. H84435 appears to be equally effective but has not been evaluated as extensively in this study.

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