FRENCH NATIONAL PROFICIENCY TESTING STUDY FOR THE STANDARDISATION OF THE CFU-GM PROGENITOR ASSAY

French National Agency for Medicines and Health Products Safety

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SUMMARY

The Laboratory Controls Division of the French Health Product Safety Agency (ANSM) had organized annual external quality control (EQC) of Hematopoietic Stem Cells (HSC) preparations from 2000 to 2013. From this experience, it appeared that it was necessary to prepare new technical guidelines for the clonogenic assay. Indeed the present guidelines did not give sufficient tools of validation. The analysis of data collected from the EQC allowed to produce recommendations which have been placed in public inquiry for three months in 2013. In order to evaluate these ones, a Proficiency Testing Study was conducted with 29 control laboratories of HSC. To that end, samples of fresh cord blood, culture medium and cell culture procedure were sent to participants. Three conditions were proposed: seeding 200 cells CD34+ per dish (compulsory requirement) with initial cord blood then according to laboratories practices, seeding at another cell concentration and/or after erythrocyte removal. For the reference condition, coefficients of variation are inferior to 40%, while discrepancies vary much more for the two other conditions (with a maximum value of 116.6%). In order to carry on the assessment of erythrocyte removal procedures initiated during this study, we performed additional testing of sedimentation. They have shown that this simple and fast method can be used with reliability but only in intra-laboratory conditions. Alongside, comments received during the public inquiry were studied. The answers were documented by analyzing data from EQC that showed an improvement of reproducibility of results obtained in 2011-2012. Eventually, the recommendations were adjusted according to our studies and will be evaluated by the Committee of biological products of the French Pharmacopoeia. All our results showing that the clonogenic assay can be used reliably to evaluate the quality of hematopoietic grafts should allow to base the publication of these recommendations in early 2014.

MATERIALS AND METHODS

Cell material: Downgraded Cord Blood Unit (CBU) obtained by a convention with the French Blood Etablishment.

Proficiency testing study organization for CFU-GM progenitor assay

A specific protocol for this study detailing the technical steps was prepared and proposed to the 33 French cell banks. Cell material and a medium of culture to ensure that all participants have the same batch (H4535 Stemcell Technologies™ medium) have been sent to the participating centres (D0). Transportation was done at a temperature between + 4 ° C and + 8 ° C by a qualified carrier for the delivery of biological products. Participants performed then the CFU-GM assay following a technical note specifying the terms of seeding and according to their own SOPs. Three conditions - including a mandatory one -were proposed: a seeding at a concentration of 200 CD34 + cells per dish with a total cord blood (mandatory requirement), seeding at another cell concentration (according to usual practices of the lab) and a seeding after erythrocyte removal.

CD34 and CFU-GM assays

CD34 numeration has been done by ANSM at D0 and results were transmitted to the 29 participants. ANSM performed a CFU-GM assay using samples within the series prepared for the distribution to the laboratories at D0 (qualification of the product), D1 (received by sites) and D2 (to cover delays transmission and/or analysis). Finally, the results have been collected and analysed by the ANSM.

Statistical analysis

Descriptive analysis has been done for each tested parameter and a Grubb test was used to identify extreme value.

RESULTS

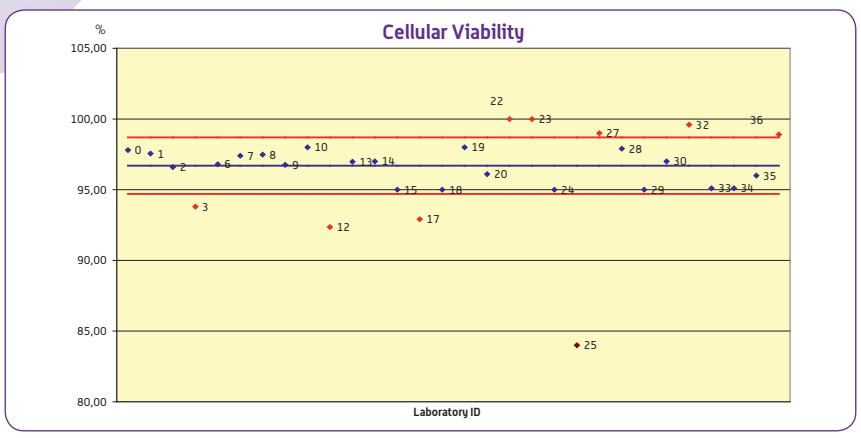
CHARACTERISTICS OF THE CBU DEDICATED TO THE PTS AT ANSM (DAY 0)

CD45+ cell number/µl	Viability	CD34+ cell number/µl	% of CD34+ cells	Microbiological control
7468	98.3%	10	0.134%	Negative

CELLULAR VIABILITY OF SAMPLES SENT TO THE PARTICIPATING LABORATORIES

n	Mean	Median	Variance	SD	CV (%)
29	96.7%	97.0%	3.8	2.0	2.0%

After transportation, viability has been determined by each laboratory at the receipt (D1) and the results are shown below. The value obtained at ANSM is mentioned for comparison (lab ID= 0).



The acceptable limits determined by the mean ± 1 standard deviation define the interval [94.7 - 98.7]

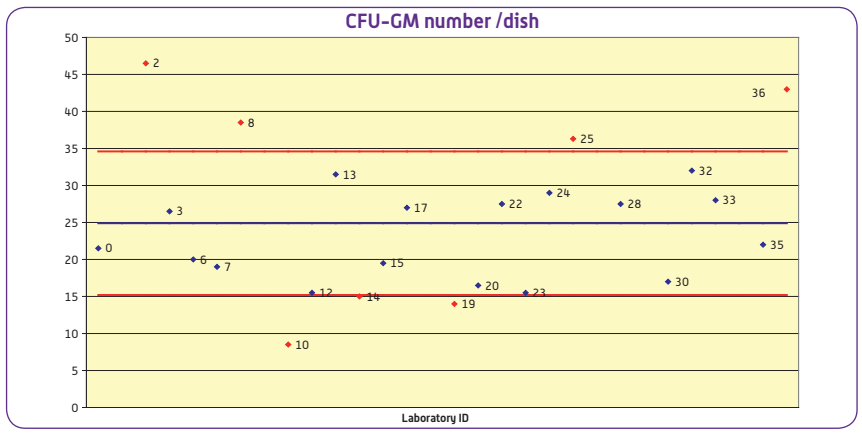
- 21 centres (70% of the participants, blue dots) are within this range.
- 8 centres (27% of participants, red dots) are outside the acceptable limits.

The value from the No. 25 centre was identified as extreme ($p < 0.05$) and was not taken into account in the descriptive analysis.

CFU-GM NUMBER OBTAINED FOR THE MANDATORY CONDITION AT 200 CD34+ CELLS /DISH

n	Mean	Median	Variance	Standard deviation	CV (%)
24	24.9	24.3	93.6	9.7	38.9%

24 laboratories answered to this variable and no value has been identified as extreme ($p > 0.05$). The number of CFU - GM colonies per dish obtained by each centre is represented below:



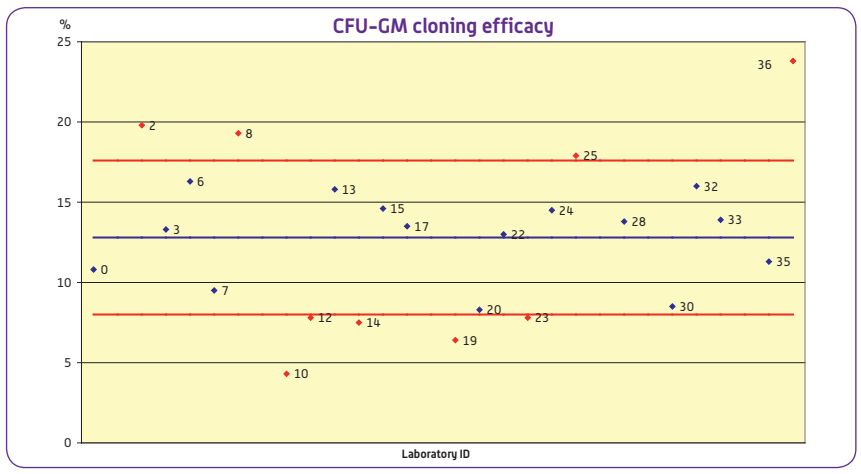
The acceptable values (mean ± 1 SD) define the interval [15.2 – 34.6]

- 17 centres (71% of the responding centres) are within this range.
- 6 centres (25% of the responding centres) are outside the acceptable limits.

CFU-GM CLONING EFFICACY

n	Mean	Median	Variance	Standard deviation	CV (%)
24	12.8%	13.4%	22.9	4.8	37.3%

24 laboratories answered to this variable and no value has been identified as extreme ($p > 0.05$). The CFU-GM cloning efficacy (CFU-GM number / CD34 number per dish *100) determined by each centre is represented below.



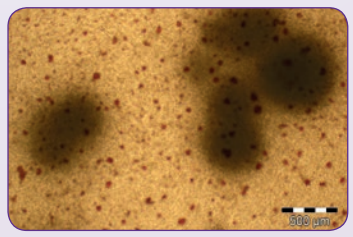
The acceptable values (mean ± 1 SD) define the interval [8.0 -17.6]

- 15 centres (62% of the responding centres) are within this range.
- 9 centres (38% of the responding centres) are outside the acceptable limits.

CONCLUSION

CV obtained for CFU-GM and cloning efficacy are near 40%. In other European PTS is often at least 50% so we can conclude that it is acceptable for this kind of assay. However, at ANSM, we obtained a significant correlation between CD34+ and CFU-GM numbers for PBSC (R^2=0.87; $p < 0.05$; n=105) with a mean cloning efficacy of 16.4 ± 5.8 with 200 CD34+/dish. Also, it seems possible in an inter-laboratories study to reduce these CV using a targeted concentration of CD34+ cells.

In this PTS, we asked to test the CBU without removing the red blood cells to keep the linearity between CFU-GM and CD34+ cells. Unfortunately, this CBU contained only 10 CD34+/µl and lead to quite high amount of red blood cells in dishes as seen on the picture. These cells could have a deleterious effect on the growth of the CFU-GM -the average for the cloning efficacy was only 12.8% instead of the 20% expected on H4535 medium- and the reading was more difficult, factors which could contribute to increase CV.



To reduce these CV, a new PTS should be performed with a miniaturized cord blood unit which contains less red cells and concentrated CD34+ cells.