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 the 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 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 Establishment.

Proficiency testing study organization for CFU-GM progenitor assay
A specific protocol for this study detailing the technical steps was prepared and proposed to 33 French cell material and medium of culture to ensure that all participants have the same batch (Hi535 Stemcell Technologies™ medium) have been sent to the participating centres (D0). Transportation was done at a temperature between +1°C to +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 and/or after erythrocyte removal.

CD34 and CFU-GM assays
CD34 number 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 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.

RESULTS

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

<table>
<thead>
<tr>
<th>CD34+ cell number/µl</th>
<th>Viability</th>
<th>CD34+ cell number/µl</th>
<th>% of CD34+ cells</th>
<th>Microbiological control</th>
</tr>
</thead>
<tbody>
<tr>
<td>7468</td>
<td>98.3%</td>
<td>10</td>
<td>0.034%</td>
<td>Negative</td>
</tr>
</tbody>
</table>

CELLULAR VIABILITY OF SAMPLES SENT TO THE PARTICIPATING LABORATORIES

<table>
<thead>
<tr>
<th>n</th>
<th>Mean</th>
<th>Median</th>
<th>Variance</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>96.7%</td>
<td>97.0%</td>
<td>3.8</td>
<td>2.0</td>
<td>2.0%</td>
</tr>
</tbody>
</table>

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].
- 31 centres (70% of the participants, blue dots) are within this range.
- 8 centres (20% 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.

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

<table>
<thead>
<tr>
<th>n</th>
<th>Mean</th>
<th>Median</th>
<th>Variance</th>
<th>Standard deviation</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>24.9</td>
<td>24.3</td>
<td>93.6</td>
<td>9.7</td>
<td>38.9%</td>
</tr>
</tbody>
</table>

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.

FU-GM CLONING EFFICACY

<table>
<thead>
<tr>
<th>n</th>
<th>Mean</th>
<th>Median</th>
<th>Variance</th>
<th>Standard deviation</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>12.8%</td>
<td>13.4%</td>
<td>22.9</td>
<td>4.8</td>
<td>37.3%</td>
</tr>
</tbody>
</table>

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) of 100 was determined by each centre and 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 it 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 FU-GM numbers for PBSC (R²=0.87; p < 0.05 ; n=105) with a mean cloning efficiency of 16.4 ± 5.8 with 200 CD34+ dishes. 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 less red cells and concentrated CD34+ cells.