Inter-laboratory method validation of CD34+ flow-cytometry assay: the experience of Turin Metropolitan Transplant Centre

Ivana Ferrero I.F.[1*], Deborah Rustichelli D.R.[1*], Sara Castiglia S.C.[1*], Loretta Gammaitoni L.G.[2*], Alessandra Polo A.P.[2*], Marisa Pautasso M.P.[3*], Massimo Geuna M.G.[4*], Franca Fagioli F.F.[1,5*]

[1] Stem Cell Transplantation Laboratory, Regina Margherita Children's Hospital, City of Health and Science of Turin, Italy; ivana.ferrero@unito.it, drustichelli@cittadellasalute.to.it, scastiglia@cittadellasalute.to.it

[2] Candiolo Cancer Institute, Fondazione del Piemonte per l'Oncologia (FPO) - IRCCS, Candiolo, Italy; loretta.gammaitoni@ircc.it, apolo@cittadellasalute.to.it

[3] Clinical Pathology Unit, University Hospital San Luigi Gonzaga, Orbassano, Italy; m61.pautasso@gmail.com

[4] Immuno-Pathology Unit, Mauriziano Umberto I Hospital, Italy; massimo.geuna@gmail.com

[5] Department of Science of Public Health and Paediatrics, University of Turin, Piazza Polonia 94, 10126 Torino, Italy; franca.fagioli@unito.it

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Author of correspondence:
Ivana Ferrero
E-mail: ivana.ferrero@unito.it;
Tel.: +39-011-3135420;
Address:
Stem Cell Transplantation Laboratory, Regina Margherita Children's Hospital, City of Health and Science of Turin, Italy

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Abstract

The Turin Metropolitan Transplant Centre (CIC 305) includes four flow-cytometry laboratories assessing quality control on hematopoietic stem cells (HSC) with different instruments and operators. Therefore, the CD34+ enumeration assay should be validated on a regular basis. We describe here the validation plan to test the inter-laboratory reproducibility of CD34+ enumeration assay, based on the risk analysis. Stabilized blood samples were analysed using Stem-Kit reagent according to manufacturer's instructions and acquired using the Beckman Coulter Navios at Regina Margherita Children's' Hospital (305-1), Beckman Coulter FC500 at Candiolo Cancer Institute FPO-IRCCS (305-2), BD Biosciences FACSLyric™ at S. Luigi Hospital (305-3), and Beckman Coulter Navios EX at Mauriziano Hospital (305-4). The ISHAGE guidelines were followed for estimating % and absolute number of CD34+ cells in single-platform method. For each sample repeatability limit (r), reproducibility error, uncertainty of reproducibility error and coefficient of variation (CV) were reported. The repeated measurements from each laboratory or instrument have a variability, expressed as reproducibility error, lower than the repeatability limit for that single parameter. The corrected reproducibility error is always lower than the repeatability limit except for the percentage value of the "low" count. The analysis of inter-laboratory variance is within the maximum acceptable variance value, and the CV of all measurements for each parameter is less than 8%, indicating low measurement variability among laboratories. Evaluating the overall data, we can conclude that the four laboratories are perfectly aligned and the results are reproducible.

Introduction

According to Joint Accreditation Committee of ISCT and EBMT (JACIE) standards, relevant and standardized assay for quantifying hematopoietic stem cell (HSC) population needs to be established and periodically validated to keep the entire process under control. The efficiency of an autologous or allogeneic HSC graft is mainly determined by the number of CD34+ cells, and their measurement by flow cytometry is an important method to assess the graft quantity (1). When HSCs are used, manipulated or not, enumeration and viability are critical information that need to be considered. Therefore, a relevant and standardized assay for quantifying the initial and final cell population needs to be established and validated (2). For many years, multi-colour flow cytometry is the technology for cell surface marker detection, viability and enumeration (3). Measurement of viable absolute counts of cells can be performed using single-platform panels recommended by International Society for Hematotherapy and Graft Engineering (ISHAGE) and JACIE standards. This includes a cell viability dye and counting beads with a lyse/no-wash preparation using commercial kits for CD34+ cells and lymphocyte subpopulations, yet without a viability dye for the latter (4-5). The challenging aspect is validating laboratory-developed flow cytometry methods, as a successful validation makes the method readily available and adapted by quality control for laboratory processing, cryopreserving or manipulating products for cell production. (2). The Turin Metropolitan Transplant Centre (CIC 305) (TMTC) is a functional program established in 2012 from the partnership of four local programs, one paediatric and three adults, on the basis of clinical and laboratory collaborations. It promotes the exchange of scientific knowledge and professional experience for clinical and research activities, implementing shared quality policies for maintaining the performance of critical activities related to HSC transplantation (HSCT). The program shares policies and aims, technical procedures and discusses on a daily basis the key performance indicators. In the TMTC, there are four flow-cytometry laboratories assessing quality control on HSCs, with different instruments and operators. For backup and emergency, a continuity plan is in place to guarantee the prompt intervention and maintenance of many services, including CD34+ evaluation. The risk that an inaccurate CD34+ enumeration may affect the efficacy of the cell-based product administered to patients need to be taken into consideration. Therefore, the CD34+ evaluation assay must be aligned among the labs, standardized and validated on a regular basis. Any change of equipment, utilities, or process should be formally documented and the impact on the validation status or control strategy assessed. A process is validated by establishing objective evidence that it consistently produces an expected endpoint or result that meets predetermined acceptance criteria. A risk assessment should be performed for each validation study to assess how critical the process is and to define the level of risk (6). The risk assessment represents a basic step to go through in the validation process. There are several methods for the assessment of the risk, such as Failure Mode and Effects Analysis (FMEA) or Failure Modes, Effects and Criticality Analysis (FMECA), methods of assessing potential failure mechanisms and their impact in the system, identifying single failure points. FMEA method can be used to assess the risk of failures and identify criticisms at every step of the process, and is widely adopted in various health care settings, including transfusion medicine (7) and HSCT (8). In this work, we describe the validation plan with relevance to the risk analysis to test the inter-laboratory reproducibility of the CD34+ enumeration method.

Materials and methods

Risk analysis

All the steps from transport of samples to the final report were carefully examined adopting a failure mode and effect analysis scheme. For the FMEA analysis, the whole process was divided into several sub-phases, and for each step, one or more different failure modes were identified. For each failure mode, a risk priority number (RPN) was calculated considering three different scores expressing severity of effects, frequency of occurrence and identifying the detection, on a scale from 1 to 4, as described in the tables 1 and 2.

Severity was multiplied for occurrence to obtain the failure risk (range 1-16). The failure risk was then multiplied for detection to obtain the RPN, the quantitative expression of each failure, ranged from 1 (1x1x1), as the "best" score, to 64 (4x4x4) as the "worst" one. RPNs rated between 1 and 9 do not need intervention; RPN between 12 and 18 need to be monitored; RPN between 24 and 32 need corrective actions; RPN between 36 and 64 require urgent corrective measures and validation plans.

To rate the occurrence of specific hazards we considered the incidence of previous failures, the presence of trained operators, the instrument maintenance process and the availability of specific standard operating procedures (SOPs).

According to the performed analysis, the most hazardous step was the analysis made by different operators with different instruments which needed a validation plan to prevent or limit errors in CD34+ detection and, as a consequence, errors in assessing quality control on HSCs.

Validation plan

To develop our work, a validation plan was prepared. This document describes the procedure and the aspects to be evaluated during the validation of the method and includes the aim of validation, the description of the method, roles and responsibilities, the acceptance criteria, and conclusion remarks.

Table 1: Failure Mode and Effects Analysis (FMEA) risk assessment. A) Calculation of failure risk; B) Calculation of risk priority number (RPN). C) Remediation plan. RPNs rated between 1 and 9 do not need intervention; RPN between 12 to 18 need to be monitored; RPN between 24 to 32 need corrective actions; RPN between 36 to 64 require urgent corrective measures and validation plan.

A

				OCCURENCE			
				LOW	MODERATE	PROBABLE	VERY PROBABLE
				FEW FAILURES	OCCASIONAL FAILURES	FAILURES ALMOST CERTAIN	VERY HIGH OCCURENCE
				1	2	3	4
SEVERITY	LOW	NO EFFECT, NO DANGER	1	1	2	3	4
	MODERATE	MINOR PART AFFECTED	2	2	4	6	8
	HIGH	LOSS OF PRIMARY FUNCTION	3	3	6	9	12
	VERY HIGH	SAFETY HAZARD	4	4	8	12	16

SEVERITY X OCCURENCE = FAILURE RISK

B

				FAILURE DETECTION			
				VERY HIGH	HIGH	MODERATE	LOW
				1	2	3	4
FAILURE RISK	LOW	NO EFFECT, NO DANGER	1	1	2	3	4
			2	2	4	6	8
			3	3	6	9	12
	MODERATE	MINOR PART AFFECTED	4	4	8	12	16
			6	6	12	18	24
	HIGH	LOSS OF PRIMARY FUNCTION	8	8	16	24	32
			9	9	18	27	36
	VERY HIGH	SAFETY HAZARD	12	12	24	36	48
			16	16	32	48	64

FAILURE RISK X FAILURE DETECTION = RPN

C

RPN			
1-9	12-18	24-32	36-64
No intervention required	Need to be monitored	Corrective actions	Corrective actions and validation plan

Table 2: Definition and interpretation of Failure Mode and Effects Analysis (FMEA) failure scores.

		Description	Examples
Severity			
Low	1	No effect, no danger	The error has no effect on results
Moderate	2	Minor part affected	Temporary instrument failure
High	3	Loss of primary function	Wrong dose of the collected graft
Very high	4	Safety hazard	Wrong dose of the infused graft
Occurrence			
Low	1	Few failures	No registered cases
Moderate	2	Some occasional failures	1-6 cases/year
Probable	3	Failures almost certain	7-12 cases/year
Very probable	4	Very high occurrence	Daily occurrence
Detection			
Very high	1	Always detected	Expiration date of reagents
High	2	Probably detected	Calibration of pipettes
Moderate	3	Middle probability of detection	Reverse pipetting error
Low	4	Probably undetected	Operator error

Samples

Stabilized blood (BD TM Stem Cell Control cod. 340991, Becton Dickinson) samples were stained with Stem-Kit reagent (cod. IM3630, Beckman Coulter) according to manufacturer's instructions. As used in external quality assessment (EQA), we chose to use the BD™ Stem Cell Control, a stable control with assigned values, routinely used to monitor the immunophenotyping process for CD34+ cells. The test was repeated on LOW (lot BC0622L: CD34+/ul=11.3 [5.9-16.7], % CD34+= 0.187 % [0.099-0.275]) and HIGH (lot BC0622H CD34+/ul =29.4 [19.5-39.3], % CD34+= 0.472 % [0.314-0.630]) samples.

Antibodies

All antibodies belonged to Stem-kit reagents: CD45 FITC Isoclonic Control-PE, CD45 FITC-CD34 PE, and 7-AAD Viability Dye were used according to manufacturer's instruction.

Lyse/no-wash single-platform viable cell enumeration and instruments

The ISHAGE guidelines were followed for estimating % and absolute number of CD34+ cells in single-platform method, using the Beckman Coulter Navios at Regina Margherita Children's Hospital (CIC 305-1), Beckman Coulter Navios at Candiolo Cancer Institute FPO-IRCCS (CIC 305-2), BD Biosciences FACSLyric™ at S. Luigi Hospital (CIC 305-3), and Beckman Coulter Navios EX at Mauriziano Hospital (CIC 305-4).

The count was performed by all four laboratories once on the LOW control, and once on the HIGH control for two consecutive days, for two weeks.

Statistical analysis

For each analysis repeatability limit (r), reproducibility error, uncertainty of reproducibility error and coefficient of variation (CV) were reported. **The reproducibility error** is the difference between the highest and lowest value measured for each parameter by each laboratory and describes the maximum variability of precision using different instruments and different operators. **Repeatability limit** (r) is the maximum acceptable deviation from the mean and it is lower than standard deviation. **The uncertainty of reproducibility** of error is the sum of the errors deriving from all the components of the test (pre-analytical phase, analytical phase, analysis phase), considered intrinsic to the measurement of that parameter. The **repeatability limit (r)** and the reproducibility error were used to compare test results within and between laboratories (9). **The coefficient of variation (CV)** is the ratio between the standard deviation and the mean and shows the extent of variability in relation to the mean. The higher the CV, the greater the dispersion. The **variance** was calculated for all the values of each laboratory and was used to calculate the **inter-laboratory variance.** The **maximum inter-laboratory variance** was the squared difference between the maximum and minimum values obtained by all laboratories.

The acceptance criteria were:

1) Reproducibility error lower than *r;*
2) CV%≤10%,
3) Inter-laboratory variance < maximum inter-laboratory variance.

Results

Risk analysis

In the validation plan, three main phases were identified: pre-analytical phase (transport of samples), processing and analysis phases, as reported in table 3.

For each step, checkpoints, criticisms and failures have been identified, and severity, occurrence and detection were assigned to each step to calculate the RPN. We found RPN ranged from 1 to 16 in most steps, where corrective actions are not requested, even if monitoring of the failure events is always recommended; while the data analysis and reporting phases reached a RPN of 36 needing specific action plans as a measure to prevent possible failures. These steps are the most difficult steps to keep under control, influenced by the interpretation of the data and by inter-laboratory reproducibility, which has been validated.

Method validation

The absolute CD34+ count was performed by the ISHAGE method on the same starting material with different instruments, operators and reagents. Counting has been performed on stabilized peripheral blood samples of known titres of LOW level (low CD34+ content) and HIGH level (high CD34+ content) by all four laboratories for two consecutive days and repeated in the same way the following week. The results obtained by four laboratories for low and high control was in the range indicated by BD TM Stem Cell Control. The repeated measurements (in percentage and absolute value) from each laboratory (or for each instrument) have a variability, expressed as reproducibility error, systematically lower than the (r) for that single parameter (low: 0.0421 for CD34+%, 2.3121 for CD34+/ul; high: 0.1026 for CD34+%, 3.6249 for CD34+/ul). The corrected reproducibility error, which represents the reproducibility error subtracted from the uncertainty of reproducibility, is always lower than (r) (low: 0.0427 for CD34+%, 2.2549 for CD34+/ul; high: 0.0854, for CD34+%, 3.1654 for CD34+/ul) except for the percentage value of the "low" count where it is slightly higher (0.0427 with r = 0.0421). The analysis of inter-laboratory variance (low: 0.0002 for CD34+%, 0.3343 for CD34+/ul; high: 0.0011 for CD34+%, 0.6025 for CD34+/ul) is within the maximum acceptable variance value (low: 0.0009 for CD34+%, 2.5122 for CD34+/ul; high 0.0036 for CD34+%, 4.9506 for CD34+/ul). In addition, the CV of all measurements for each parameter analysed is less than 8%, indicating low measurement variability among laboratories. Overall results were summarized in table 4.

Table 3: Validation plan

PROCESS STEPS	MATERIALS	INSTRUMENTS	CHECK POINTS	CRITICISMS	FAILURES	SEVERITY	POTENTIAL CAUSES	OCCURENCE	FAILURE RISK	CONTROLS	DETECTION	RPN
1. Pre-analytical phase												
Transport of samples	PB, PBMC, BM	Courier Transport box	Check on integrity of sample	Temperature during transport	Deterioration of sample	3	Courier not compliant Temperature not compliant	1	3	Training of courier	1	3
				Delay in delivery	Delay in processing		Communication errors Problems during transport	2	6	Training of courier	1	6
2. Processing												
Evaluation of [WBC]	PB/BM	Haematology analyser	Cell concentration	Cell concentration not compliant for staining	Results not accurate	2	Instrument failure	2	4	EQA	1	4
	PBMC											
Labelling	PBS, Monoclonal antibodies	Pipette, tips	Control sample	Errors in staining	Results not accurate	3	Pipette failure	2	6	Annual calibration	2	12
				Reverse pipetting			Operator not trained/ distraction			Competence evaluation, training and retraining		
Acquisition	Lysis solution	Pipette	Stem Count Fluorospheres	Lysis solution not compliant	Results not accurate	3	Pipette failure	2	6	Annual calibration	1	6
	Fluorosphere			Reverse pipetting			Operator not trained/ distraction			Competence evaluation, training and retraining		
3. Analysis												
Data analysis and reporting	Software	Cytometer	Daily QC	OOS	Errors in results	4	Cytometer failure	2	8	Periodic OQ/PQ	2	16
			Isotype control	Gating strategy		4	QC reagent expired	2	8	Reagent control	1	8
							Operator not trained/ distraction			Competence evaluation, training and retraining		
			Results	Inter-laboratory reproducibility	Results not accurate	4	Operators/ Instruments/ reagents	3	12		3	36

Table 4: Summary of results

	BD TM Stem Cell Control LOW		BD TM Stem Cell Control HIGH	
	CD34+ %	**CD34/ul**	**CD34+ %**	**CD34/ul**
Sample Range	0.099-0.275	5.9-16.7	0.314-0.630	19.5-39.3
Mean	0.1950	12.2690	0.4705	29.6510
SD	0.0150	0.8258	0.0366	1.2946
CV	7.7148	6.7305	7.7856	4.3662
Repeatability limit (r)	0.0421	2.3121	0.1026	3.6249
Reproducibility error	0.0600	3.1700	0.1200	4.4500
Uncertainty of reproducibility error	0.0173	0.9151	0.0346	1.2846
Corrected reproducibility error	0.0427	2.2549	0.0854	3.1654
Inter-laboratory variance	0.0002	0.3343	0.0011	0.6025
Maximum acceptable variance	0.0009	2.5122	0.0036	4.9506

Discussion

FACT–JACIE standards have evolved over time, with scheduled review and revision based on the rapidly changing fields of HSCT and cellular therapy. Quality management vision also changed. The systems were primarily based on a "safety first approach.", while nowadays it is moving towards ensuring that "as many things as possible go right" (10). When HSCs are used, CD34 flow cytometry is the gold standard for stem cell enumeration both in peripheral blood (PB) and in the stem cell product (apheresis, bone marrow or cord blood) prior to transplantation. It requires well-trained and experienced operators and is a technique that can be technically challenging (11). The number of infused viable CD34+ cells strongly correlates with the time to hematologic recovery of the patient (12). Therefore, in case of autologous transplantation, the number of CD34+ cells in PB is a guide to the start of collection, the blood volume to be processed, and the number of apheresis procedures to be performed. Successful mobilization of PB stem cells and adequate stem cell collection are of critical importance. Doses of 2×10^6 to 5×10^6 CD34+ cells/kg body weight are associated with more rapid engraftment and a lower probability of graft failure (13). Low CD34+ cell doses are associated with increased cost and worse outcome after tandem autologous stem cell transplantation in patients with relapsed or refractory germ cell tumours. Moreover, in the autologous setting, enumeration of viable CD34+ cells at the time of infusion becomes particularly relevant in patients in whom stem cell mobilization has been problematic and/or in which a total amount of stem cells was collected which was borderline to ensure a safe transplant procedure (i.e. 1.0-2.5 × 106 CD34+/kg) (14). Stem cell laboratories should have policies and procedures that address interruption in routine activity due to equipment failure or other emergency that may occur, so that such interruptions do not adversely affect cellular therapy products, critical supplies, and processes (1). In a program that involves the sharing of processes and procedures, the availability of different services and laboratories is a strength. However, the risk that an inaccurate CD34+ enumeration may affect the efficacy of the cell-based product administered to patients need to be taken into consideration. The inter-laboratory variability and reproducibility of the method is even more important when talking about cryopreserved cells. CD34+ cell counting techniques is well standardized on fresh samples, whereas the cytometry analysis of thawed samples is still controversial, and no validated techniques are yet available (15,16). In this context, we set up a risk analysis to test the inter-laboratory reproducibility of the CD34+ enumeration method, among the four TMTC laboratories, analysing the repeatability limit (r), the reproducibility error, the uncertainty of reproducibility error and the CV (CV). As reported since many years, participation in external EQA proficiency testing improve the accuracy of the method. EQA participation coupled with effective laboratory monitoring and remedial action is strongly associated with improved laboratory accuracy, and therefore with more appropriate patient treatment decisions (17). All the TMTC laboratories participate in external quality assessment that is an integral part of laboratory work and mandatory when the results have a clinical application. As used in EQA, we chose to use the the BD™ Stem Cell Control, a stable control, with assigned values routinely used to monitor the immunophenotyping process for CD34+ cells, and to ensure that the lab processes and operations run efficiently and guarantees the production of accurate and reproducible results. BD™ Stem Cell Control has been adopted by several Transfusional Centers around Europe. In the last 12 months this specific Quality Control is in use in 310 Laboratories in Europe (65 of these Laboratories are located in Italy), Middle East and Africa. The low values of the reproducibility errors indicated high reproducibility between laboratories. Moreover, the low inter-laboratory variability was demonstrated also by CV%< 10%. Evaluating the overall data, we can assume that the four laboratories are perfectly aligned and the results are reproducible. The statistical data obtained in this validation work, led us to demonstrate the crucial importance an accurate risk analysis shared by all the laboratories involved. The standardization of the method in use among the different laboratories of the TMTC allows the optimization of the processes, and guarantees the continuity of the services even in situations of emergency and disasters. As already reported, the use of a common standardized protocol, targeted training and external quality assessment significantly reduces intra- and inter-laboratory CD34+ cell count variation (18).

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Author Contributions:

IF supervised the work and was responsible of writing and revision of the paper; DR, SC were responsible for designing and writing the validation protocol, analysing the samples and contributed to write the paper; LG, AP, MP were responsible for analysing the samples and contributed to write the paper; MG was responsible for extracting and analysing data and interpreting results; FF was responsible of critical reading of the paper.

Competing Interests

The authors declare that they have no competing interests in relation to the work described.

Data Availability Statement

Raw data supporting the findings of this study are available from the corresponding author upon reasonable request.

References

1. Wuchter P. Processing, Cryopreserving and Controlling the Quality of HSCs. In: The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies. Cham (CH), Springer. 2019;. DOI: 10.1007/978-3-030-02278-5 17

2. Mfarrej B, Gaude J, Couquiaud J, Calmels B, Chabannon C, Lemarie C. Validation of a flow cytometry-based method to quantify viable lymphocyte subtypes in fresh and cryopreserved hematopoietic cellular products. Cytotherapy 2021;23:77-87. https://doi.org/10.1016/j.jcyt.2020.06.005.

3. Jaye DL, Bray RA, Gebel HM, Harris WAC, Waller EK. Translational applications of flow cytometry in clinical practice. J Immunol 2012;188:4715-9. https://doi.org/10.4049/jimmunol.1290017.

4. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International society of hematotherapy and graft engineering. J Hematother 1996;5:213 26. https://doi.org/10.1089/scd.1.1996.5.213

5. Keeney M, Chin-Yee I, Weir K, Popma J, Nayar R, Sutherland DR. Single platform flow cytometric absolute CD34+ cell counts based on the ISHAGE guidelines. Cytometry 1998;34:61-70.

6. Monteleone R and Klarmann D. Qualification and Validation. In Quality Management and Accreditation in Hematopoietic Stem Cell Transplantation and Cellular Therapy: The JACIE Guide. Chapter 5, Springer 2021. ISBN 978-3-030-64491-8 ISBN 978-3-030-64492-5 (eBook).

7. Najafpour Z, Hasoumi M, Behzadi F, Mohamadi E, Jafary M, Saeedi M. Preventing blood transfusion failures: FMEA, an effective assessment method. BMC Health Serv Res 2017;17:453. https://doi.org/10.1186/s12913-017-2380-3

8. Teofili L, Bianchi M, Valentini CG, Bartolo M, Orlando N, Sica S. Validation plan of bone marrow collection, processing and distribution using the failure mode and effect analysis methodology: a technical report. Cytotherapy 2022;24:356-364. https://doi.org/10.1016/j.jcyt.2021.10.005

9. Cool P, Ockendon M. Stats Book. http://countcool.com. ISBN 978-0-9560215-7-1

10. Snowden JA, McGrath E, Orchard K, Kröger N, Sureda A, Gratwohl A. Visions for a JACIE Quality Management System 4.0. Bone Marrow Transplant 2021;56:2876-2881. https://doi.org/10.1038/s41409-021-01467-8

11. Whitby A, Whitby L, Fletcher M, Reilly JT, Sutherland DR, Keeney M, Barnett D. ISHAGE protocol: are we doing it correctly? Cytometry B Clin Cytom 2012;82:9-17. https://doi.org/10.1002/cyto.b.20612

12. Schmitz N, Barrett J. Optimizing engraftment source and dose of stem cells. Semin Hematol 2002;39:3-14. https://doi.org/10.1053/shem.2002.29245.

13. Hyder MA, Goebel WS, Ervin KD, Schwartz JE, Robertson MJ, Thakrar TC, Albany C, Farag SS. Low CD34+ Cell Doses Are Associated with Increased Cost and Worse Outcome after Tandem Autologous Stem Cell Transplantation in Patients with Relapsed or Refractory Germ Cell Tumors. Biol Blood Marrow Transplant. 2018;24:1497-1504. https://doi.org/10.1016/j.bbmt.2018.01.032

14. Lanza F, Campioni D, Hellmann A, Milone G, Wahlin G, Walewski J, et al. Editorial Transfusion and Apheresis Science 59 (2020) 102785 2. Individual quality assessment of autografting by probability estimation for clinical endpoints: a prospective validation study from the European group for blood and marrow transplantation. Biol Blood Marrow Transplant 2013;19:1670-6. https://doi.org/10.1016/j.bbmt.2013.08.005

15. Fournier D, Lewin A, Simard C, Trépanier P, Néron S, Ballerini L, Codinach M. Multi-laboratory assay for harmonization of enumeration of viable CD34+ and CD45+ cells in frozen cord blood units. Cytotherapy 2020;22:44–51. https://doi.org/10.1016/j.jcyt.2019.10.009

16. Lanza F, Saccardi R, Seghatchian J. New horizons on stem cell cryopreservation through the artificial eyes of CD34+, using modern flow cytometry tools. Transfus Apher Sci 2020;59:102785. https://doi.org/10.1016/j.transci.2020.102785

17. Bainbridge J, Rountree W, Louzao R, Wong J, Whitby L, Denny TN, Barnett D. Laboratory Accuracy Improvement in the UK NEQAS Leucocyte Immunophenotyping Immune Monitoring Program: An Eleven-Year Review via Longitudinal Mixed Effects Modeling. Cytometry B Clin Cytom 2018 Mar;94(2):250-256. https://doi.org/10.1002/cyto.b.21531

18. Barnett D, Granger V, Kraan J, Whitby L, Reilly JT, Papa S, Gratama JW. Reduction of intra- and interlaboratory variation in CD34+ stem cell enumeration using stable test material, standard protocols and targeted training. DK34 Task Force of the European Working Group of Clinical Cell Analysis (EWGCCA). Br J Haematol. 2000;108:784-92. doi: 10.1046/j.1365-2141.2000.01932.