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Estimation of the allowable total error of the absolute CD34+ cell count by flow cytometry using data from UK NEQAS exercises 2004–2024

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Abstract

Objectives: The knowledge of the measurement uncertainty (MU) of a diagnostic laboratory test is essential to keep the reliability of laboratory results under control, is requested by regulatory bodies, and for the clinician to be aware of the grey zone of variability around the reported values. The calculation of the percent allowable total error (%aTE) defines the levels of acceptable and optimal MU for each measurand. The CD34+ hemopoietic precursor cell level in blood, as a flow cytometric measurand, still lacks reliable MU and %aTE indicators.

Methods: %aTE of the absolute count of CD34+ cells in stabilized peripheral blood has been evaluated using a UKNEQAS database of 69,294 valid results entries from the Stem Cell Enumeration EQA/PT Programme over the last 20 years. The state-of-the-art (SOTA) desirable performance achievable by 80 % of participants and the optimal performance by the best laboratories were calculated at four levels of absolute CD34+ cell counts, from 0 to 10 to >50 cells/μL.

Results: Double platform users displayed worse %aTE as compared to single platform users in both periods, with a general trend to improvement with time. Single platform users in the 2014–2024 decade performed best, with a flat % aTE trend over the years. The SOTA-based %aTE were calculated for each method and every decision-making cell level, showing relatively narrow ranges.

Conclusions: Our EQA/PT study with stabilized peripheral blood CD34+ cell suspensions reliably estimated the %aTE of the absolute CD34+ cell count, mostly related to the purely analytical variability and devoid of the preanalytical interferences caused by the decay of fresh samples.

Keywords: absolute count; allowable total error; CD34+ cells; EQA/PT; measurement uncertainty; SOTA

Introduction

The ISO15189:2022-12 international standard for clinical laboratories at section 7.3.4 states for any analyte or measurand the need to define the degree of measurement uncertainty, the biological reference intervals, and the values that constitute thresholds and limits for clinical decisions. The ISO/TS 20914:2019 document provides the practical guidance to calculate and establish such important laboratory parameters [1–3].

The knowledge of the measurement uncertainty (MU) of a diagnostic laboratory test is essential for the laboratory to keep the reliability of its results under control, is requested by regulatory bodies, and for the ordering clinician to be aware of the ‘grey zone’ of variability around the reported values.

A major contributor of the MU is the total analytical error (TE) related to the measurement of a certain analyte, a technical parameter generated by the summation of all the involved analytical and extra-analytical variables. Another large component of the MU is the biological variability (BV) of the analyte itself. While the components of TE are amenable of a continuous improvement through practice and technological advancements, within-subject and between-subject BV cannot be analytically improved [4, 5].

Therefore, for every analyte of clinical significance efforts have to be made to define the acceptable limits of the variation around the ‘true’ target value, calculating the allowable Total Error (aTE), in order to prevent the misdiagnosis or the misclassification of patients and provide confidence when taking necessary clinical decisions [6, 7].

Hundreds of common clinical laboratory measurands have been already extensively studied to define the MU, BV and aTE parameters, since their value ranges are reasonably

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stable and predictable. Open-source reference tables have been published by CLIA and other expert groups [8, 9], with several cases also taking into account the BV values of percent and absolute lymphocyte subset measurements [10, 11].

Percent aTE calculations are accomplished using the classical formulae devised by C.G. Fraser, defining the minimum, desirable and optimum ranges of total allowable imprecision and the goal of analytical bias [7, 12, 13]. Such formulae require however the previous knowledge of both within- and between-subject BV for the measurand of interest [5].

Published data on CD34+ hematopoietic precursor cells (HPC) BV to be applied to the Fraser's formulae are unfortunately not yet available, and just a few studies have been published on the baseline CD34+ cell levels in the peripheral blood of healthy subjects by flow cytometry (FCM) [14]. In normal subjects the peripheral blood level of CD34+ HPCs ranges typically from 0 to about 10/μL or <0.1 % of leukocytes, and when the level raises stably above 10 cells/μL, the presence of some derangement in the bone marrow becomes likely [15].

International efforts have been made during the last three decades to standardize the CD34+ measurements and to increase their robustness in the clinical practice, thanks to the advent of FCM boolean gating, the development of the ISHAGE guidelines in 1996 and the worldwide availability of external quality assurance systems [16].

Defining the %aTE for CD34+ HPC enumeration in the autologous or allogeneic transplantation setting is of the utmost importance, but is particularly challenging, because the HPC absolute levels are not stable and predictable variables, but may vary by a factor of several hundreds during the various phases of the mobilization process and cell collection. In the absence of published BV data, as for newly devised analytes or drug dosages, alternative or in house methods to define the aTE must be sought for, which can be difficult [1]. Moreover, the various matrices in which CD34+ HPCs are measured, such as peripheral blood (PB), bone marrow, fresh and thawed cord blood, fresh and thawed apheresis require the application of specific technicalities and further complicate these assessments [16–21].

At present only one valuable study has been published on the evaluation of %aTE in CD34+ HPC enumeration using data from a Spanish external quality assessment program and employing the 'state of the art' (SOTA) methodology [6] with the distribution of fresh samples [22].

Taking inspiration from this pioneer study we have made an extensive evaluation of %aTE of the measurement of CD34+ cells absolute levels in stabilized PB preparations distributed worldwide by the United Kingdom for National External Quality Assessment – UK NEQAS – CD34+ Stem Cell Enumeration EQA/PT Programme over the last 20 years, from 2004 to 2024. This involved calculating the SOTA-based %aTE values, defined as the desirable performance level that can be achieved by 80 % of participating laboratories and the optimal performance displayed by the best performers, as clustered in the upper quartile of results distribution, with a clear trend of improvement with time.

Materials and methods

Data collection

In the fully ISO 17043 accredited UK NEQAS CD34+ Stem Cell Enumeration Programme two vials with 1 mL of stabilized PB samples containing unknown amounts of CD34+ cells were regularly distributed from four to six times a year to international participants, starting from April 1996. For the present study two more recent consecutive 10-years periods were selected, from January 2004 to March 2014 (180 participants), and from May 2014 to August 2024 (up to 370 participants), to better reflect the current practice.

The 2004–2024 raw results database contained 77,356 entries from 246 exercises. Participants used multicolor flow cytometers and the ISHAGE protocol either in double platform (DP) employing FCM for CD34+ cell percentage and an automated blood counter to obtain the absolute total white blood cell count, or in single (SP) platform to obtain the absolute CD34+ cell level directly from the FCM using calibrated counting beads.

For each exercise, outliers were eliminated in two steps: first manually by eliminating void entries, typing and clerical errors, grossly abnormal inputs like 0 or 9,999, then the genuine measurement outliers were eliminated using the Python software and the interquartile range method [23, 24]. The medians of each exercise were then calculated. After the cleaning procedure that eliminated 10.43 % of the raw data, the database to be analyzed contained globally 69,294 valid entries.

The first observation period (120 exercises from January 2004 to March 2014) generated a total 28,522 entries and was characterized by a rather large fraction of participants still using DP (globally 33.1 %) but tapering from 102 in 2004 to 47 in 2014, and generating 9,456 valid entries. SP users increased from 64 to 236 during the same observation period, generating 19,066 entries. The data and the analyses from the 2004–2014 period were then kept separated between DP and SP users.

The second observation period (126 exercises from May 2014 to August 2024) generated a total 33,906 SP entries and was characterized by an ever-increasing group of SP users (from 236 in 2014 to 303 in 2024), while the DP group progressively thinned. Despite the clearcut recommendations about using SP in the FCM guidelines for CD34+ cell enumeration [21, 25], however, DP users in the 2014–2024

decade still represented about 15 % of the participants and generated 6,866 valid entries. Data from DP users in the 2014–2024 decade were separately analyzed just for sake of completeness.

Data analysis

Exercise results were classified according to the respective medians into four levels of absolute CD34+ cells/μL.

- Level A from 0 to 10 CD34+ cells/μL
- Level B from 10.1 to 25 CD34+ cells/μL
- Level C from 25.1 to 50 CD34+ cells/μL
- Level D >50.1 CD34+ cells/μL, respectively

Level A data reflect the laboratory practice with normal PB and the diagnostic monitoring of myelodysplastic and myeloproliferative disorders [26–28], whereas Levels B, C and D represent the CD34+ cell levels variably used by clinicians to trigger the start of HPC collection by leukapheresis both in the autologous and the allogeneic settings [29, 30]. Table 1 summarizes the composition of the resulting dataset.

The valid results of each exercise were then used to calculate the SOTA-Based %aTE against the respective median, according to the following formula [31]:

SOTA-Based %aTE = [Absolute Value (Reported Result–Median)/Median] × 100

The %aTE median value for each exercise and anaysis technique was recorded. The %aTE values were then pooled into the respective groups of CD34+ cell levels, the data were checked for normality using the Shapiro-Wilk test and finally sorted in ascending order to generate the cumulative distribution curves. The median and interquartiles of each distribution were calculated. The cases with a calculated % aTE >500, a value still indicating an abnormal result about 6 times higher than expected despite the removal of outliers, were excluded. The data analysis was accomplished with Python, using MatPlotLib and Pandas libraries.

For each cumulative distribution, by intercepting the sigmoid curve at Y=80 %, the corresponding X values of % aTE indicate the allowable error achievable by 80 % of laboratories for each considered CD34+ cells/μL level (i.e. the desirable SOTA performance level).

For each cumulative distribution, the median %aTE calculated on the results relative to the upper quartile of valid data represents the optimal performance that can be theoretically achieved by all participants as an improvement goal [6] (i.e. the SOTA-Based Optimal %aTE).

Results

Changes in protocol usage over time

In the 2004–2014 decade participants declared the use of in-house ISHAGE protocol (78.8 %), Beckman Coulter Stem Kit (9.9 %), in-house Milan protocol (7.0 %) and Becton Dickinson Procount (4.3 %). In the 2014–2024 decade the use of in-house ISHAGE protocol decreased (61.7 %), the ISHAGE-based Becton Dickinson Procount and Stem Cell Kit increased (23.1 %), as well as the Beckman Coulter Stem Kit (14.3 %), while the in-house Milan protocol tapered to a residual 0.9 %.

Among the 120 exercises of the 2004–2014 period the lowest median absolute CD34+ cell level tested was 2.2/μL, with just four samples ranging <5/μL (3.3 %), while the highest level was 141/μL. Among the 126 exercises of the 2014–2024 period the lowest absolute CD34+ cell level tested was 0.6/μL, with 11 samples ranging <5/μL (8.7 %), while the highest level was 184/μL.

Comparison of single platform and dual platform techniques

Figure 1 represents all the %aTE cumulative distributions of the four absolute CD34+ cells/μL levels (A, B, C and D,

Table 1: Composition of the data groups stratified according to analysis technology (DP, SP), observation period (2004–2014 and 2014–2024) and four absolute CD34+ cell levels/μL (A, B, C and D, respectively). A total of 69,294 valid entries were included in the analysis.

	2004–2014 dual platform (Exercises, n) entries	2004–2014 single platform (Exercises, n) entries	2014–2024 dual platform (Exercises, n) entries	2014–2024 single platform (Exercises, n) entries
Level A (0–10 cells/μL)	(19) 1712	(19) 2,820	(26) 1,544	(26) 7,148
Level B (10.1–25 cells/μL)	(37) 3,258	(37) 4,857	(53) 2,859	(53) 14,401
Level C (25.1–50 cells/μL)	(29) 2057	(29) 5,193	(28) 1,518	(28) 8,140
Level D (>50.1 cells/μL)	(35) 2,429	(35) 6,196	(19) 945	(19) 4,217
Total	(120) 9,456	(120) 19,066	(126) 6,866	(126) 33,906

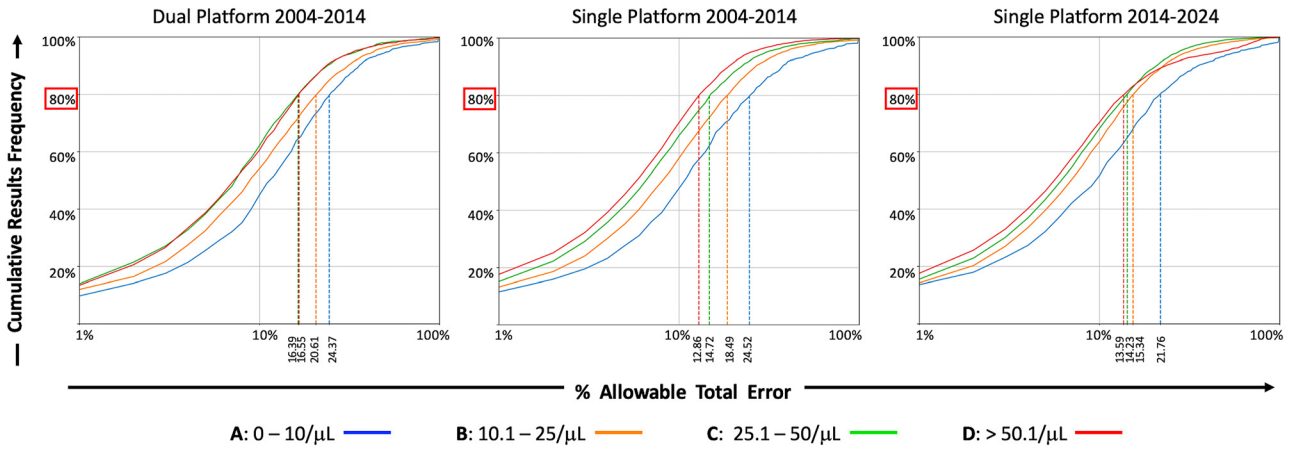

Figure 1: The %aTE cumulative distributions of the four absolute CD34+ cells/µL levels (A, B, C and D, respectively, represented in colors) in the three main groups of data are shown. The respective %aTE values obtained by intercepting the curves with Y=80 % are indicated on the X axis. The lowest CD34+ cells/µL levels show the highest %aTE values in all instances.

respectively) in the three more significant groups of data. The respective %aTE values obtained by intercepting the curves with Y=80 % are indicated. The SOTA-based %aTE showed a trend to the reduction with the increase of CD34+ cell levels. The lowest %aTE values are associated with SP analyses, and improved during the two observation periods.

Table 2 summarizes the quantitative results, including the median and interquartiles of the cumulative distributions, the SOTA-Based Desirable %aTE achievable by 80 % of the laboratories and the SOTA-Based Optimal %aTE, representing the highest level of analytical performance theoretically achievable.

The DP and SP %aTE for the lowest CD34+ cell levels was nearly the same, while DP appeared to perform progressively worse with the increasing of the CD34+ cell levels. The SP %aTE in the 2014–2024 decade showed a further improvement.

As expected, the overall %aTE progressively reduces with the increase of the CD34+ cell levels in all instances. The marked difference between desirable and optimal %aTE levels summarized in Table 2 shows that a reduction of about 10 times of the measurement uncertainty is potentially achievable, as demonstrated by the best performing laboratories.

Supplementary Figure 1 illustrates more clearly the direct comparison between DP and SP %aTE in the 2004–2014 period. The overall performance of the two techniques was remarkably similar in the A level group, while for increasing CD34+ cells/µL levels the DP laboratories showed a progressively worsening performance.

Table 2: The medians and interquartiles of all cumulative distributions, the SOTA-based desirable %aTE achievable by 80 % of the laboratories and the SOTA-based optimal %aTE values are summarized for DP 2004–2014 and SP 2004–2014/2014–2024. All the %aTE parameters progressively decrease with the increasing of the CD34+ cells/µL levels.

		Median CD34+ cells/µL	(Exercises, n) entries, n	Median %aTE (interquartiles)	SOTA-based desirable %aTE (achievable by 80 % of labs)	SOTA-based optimal %aTE (upper quartile of labs)
Dual platform 2004–2014	A	0–10.0	(19) 1,712	12.47 % (5.91–22.50)	± 24.37 %	± 2.50 %
	B	10.1–25.0	(37) 3,258	9.81 % (4.54–18.78)	± 20.61 %	± 2.12 %
	C	25.1–50.0	(29) 2,057	8.31 % (3.70–15.03)	± 16.55 %	± 1.73 %
	D	>50.1	(35) 2,429	8.15 % (3.74–15.29)	± 16.39 %	± 1.84 %
Single platform 2004–2014	A	0–10.0	(19) 2,820	11.57 % (5.26–21.97)	± 24.52 %	± 2.27 %
	B	10.1–25.0	(37) 4,857	8.94 % (4.12–16.91)	± 18.49 %	± 1.87 %
	C	25.1–50.0	(29) 5,193	7.50 % (3.46–13.78)	± 14.72 %	± 1.56 %
	D	>50.1	(35) 6,196	6.77 % (2.96–12.26)	± 12.86 %	± 1.43 %
Single platform 2014–2024	A	0–10.0	(26) 7,148	10.53 % (4.40–19.54)	± 21.76 %	± 1.68 %
	B	10.1–25.0	(53) 14,401	7.88 % (3.64–14.37)	± 15.34 %	± 1.68 %
	C	25.1–50.0	(28) 8,140	7.09 % (3.25–13.20)	± 14.23 %	± 1.63 %
	D	>50.1	(19) 4,217	6.57 % (2.94–12.50)	± 13.59 %	± 1.42 %

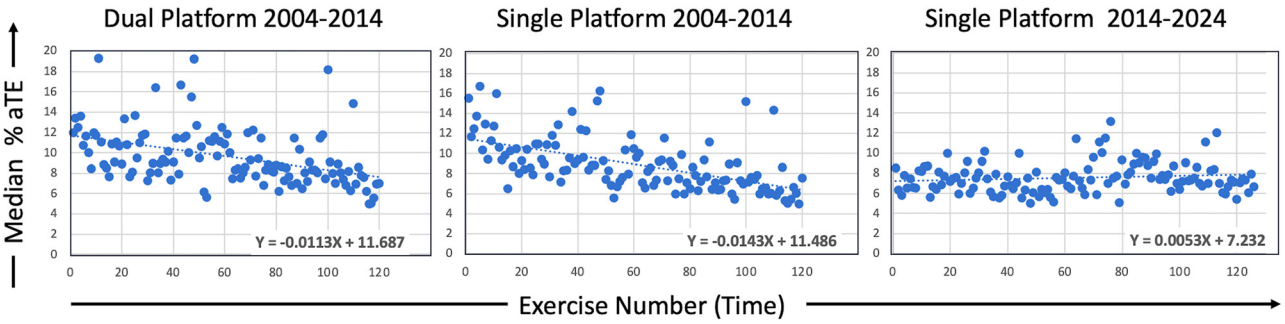

Figure 2: Trend of the median %aTE with time in the three main groups of analyzed data. While performance improvements could be observed during the 2004–2014 decade, after 2014 the overall performance remained stable with a median aTE around 7 %.

Supplementary Figure 2 shows the comparison between SP %aTE in the 2004–2014 and 2014–2024 periods. The overall SP performance seemed to improve only slightly in the more recent period, especially for the two lowest CD34+ cells/μL levels, while no significant improvements could be noted for the two highest levels.

Supplementary Table 1 shows the performance of the residual DP users in the 2014–2024 decade. The %aTE parameters showed no significant improvement as compared to the 2004–2014 decade, and were significantly worse than the corresponding SP data during the same observation period.

Figure 2 shows the trend of the median %aTE with time in the three main groups of analyzed data. A progressive and parallel reduction of median %aTE was observed for both DP and SP in the 2004–2014 period, with just slightly higher figures for DP. Conversely, the overall performance of SP laboratories in the 2014–2024 period did not further improve with time, with aTE remaining stably around about 7 % over the years.

No instrument- or manufacturer-related bias could be noted with %aTE values, and this is in line with the remarkably overlapping performance data between the two major providers of CE-IVD certified FCM systems that can be inferred from the UKNEQAS scheme reports. No results from participants using SP volumetric instruments in CD34+ counting are recorded at present.

According to our results, a reliable estimate of the 'grey zone' around a given reported result of absolute CD34+ cells/μL using the current SP technique can be defined. The grey zone varies according to the acceptability criteria used: the SOTA-Based desirable %aTE may range from 21.76 % for levels around 10 CD34+ cells/μL to 13.59 % for levels around 50 CD34+ cells/μL. Conversely, the SOTA-Based optimal % aTE, reflecting the results of highest quality achievable by the best performing laboratories may range in a much narrower spectrum, from 1.68% to 1.42 % for the same CD34+ cells/μL levels, as summarized in Table 3.

Table 3: The 'grey zones' around a certain reported value of CD34+ cells/μL obtained by SP and calculated according to two different criteria of allowable imprecision are indicated. The reported levels of 10, 20 and 50 CD34+ cells/μL refer to the most commonly used thresholds to trigger an apheresis procedure.

	What a given reported result by SP may mean	
Reported CD34+ cells/μL	**According to SOTA-desirable % aTE (80 % of labs)**	**According to SOTA-optimal %aTE (upper quartile of labs)**
10	7.83–12.17	9.84–10.16
20	16.94–23.06	19.68–20.32
50	43.25–56.67	49.3–50.7

Discussion

The absolute level of CD34+ cells in a biological matrix represents an example of a peculiar, multi-faceted FCM analyte with several different applications in multiple clinical settings, and endowed with a great decision-making impact.

The CD34+ cell enumeration by FCM is a fully mature technology, taking advantage from internationally established guidelines [21], the availability of standard reference materials and calibration curves. An evaluation of the laboratory practice should nowadays focus on desirable and optimal performance levels only, leaving acceptable levels aside.

The measurement of CD34+ cells in the PB of steady-state individuals is a typical rare event FCM analysis, aimed at identifying bone marrow abnormalities leading to myelodysplastic syndrome, myelofibrosis and myeloproliferative disorders when levels exceed 10 CD34+ cells/μL [26–28]. Circulating and bone marrow CD34+ cells have long been assumed as 'blast' equivalents, and despite several biological limitations, the FCM enumeration of CD34+ cells proved to be a practical surrogate tool to estimate the number of myeloid blasts in hematologic malignancies, and especially

to classify different disease states and to monitor patients [32–34]. The semi-quantitative evaluation of CD34+ blasts in the peripheral blood or bone marrow of patients with acute leukemias remains however outside the scope of true quantitative assessments.

In the setting of autologous and allogeneic transplantation of CD34+ HPCs the trigger PB values to start the apheresis collection in mobilized patients and donors vary greatly according to local policies, ranging typically from 10 to 50 CD34+ cells/μL. The concentrated fresh apheresis cell suspension may contain viable CD34+ HPCs ranging from several hundreds to a few thousands per μL, whereas the respective cryopreserved preparations introduce a great deal of measurement variability after thawing, related to the high cell mortality. Similar problems can be faced in the analysis of CD34+ HPCs in the cord blood.

With such a wide range of absolute levels and diverse biological matrices the evaluation of the allowable total error of the absolute CD34+ cell enumeration is therefore particularly challenging, also due to the total lack of published data on biological variability.

In this scenario, accuracy-based EQA/PT programs have the unique ability to provide laboratorians with large datasets of unbiased measurements across laboratories and manufacturers, that can be used to generate the necessary %aTE parameters to guide the routine practice [5]. The only study so far published on the %aTE evaluation of CD34+ cells has shown a global %aTE of 27.8 % for the best performing laboratories, using fresh unstabilized samples distributed to 40 laboratories in Spain [22].

In our study we aimed at evaluating the SOTA-Based %aTE for a large number of international laboratories participating to the UK NEQAS – CD34+ Stem Cell Enumeration Programme over two consecutive 10-years periods from 2004 to 2024, using highly consistent stabilized PB preparations. We tried to calculate the SOTA-Based %aTE levels for the enumeration of CD34+ cells in hematological patients' PB (i.e., ≤10 cells/μL) and for the main relevant checkpoints of the mobilization procedure (i.e., 20, 50 and >50 cells/μL, respectively). In our analysis the highest levels provided during the exercises were between 141 and 184 CD34+ cells/μL, while the unavailability of concentrated apheresis suspensions prevented us from making evaluations for higher values.

In this context, the term 'SOTA' does not denote the use of a specific antibody mixture, instrumentation or software, rather it defines the %aTE values that can be achieved as current procedure standards by 80 % of laboratories [1], while the median %aTE of the upper quartile of participants represents the performance of the highest quality that can be virtually achieved by all as an improvement goal [6].

As compared to the study by Fernández-Luis, our results showed tighter %aTE ranges for all the considered absolute CD34+ cell levels, probably due to the type of distributed samples, that ensure stable and homogeneous levels of both total white cells and CD34+ precursors, despite the long delivery distances and times. Our findings therefore may reflect a more accurate picture of the purely analytical imprecision related to the FCM absolute CD34+ cell measurements, devoid of the unavoidable preanalytical decay related to the long-range distribution of fresh samples [35]. Our %aTE values showed a progressive narrowing with the increase of the absolute CD34+ cell level, in line with the statistical principles described by Horwitz [36]. Conversely, we were challenged by a globally higher number of outliers than in the study by Fernández-Luis (10.43 % vs. 6.4 %, respectively). This was likely due to the larger number of participants (up to 370 vs. 40), dispersed in up to 59 countries worldwide, with a frequent in-and-out turnover of laboratories, and consequently a lower degree of control and direct advice by the scheme managers.

The 2004–2014 decade showed a constant reduction of the %aTE with time for both DP and SP users, while during the subsequent decade the %aTE of SP users remained rather flat without showing further improvements. Almost all participants declared the usage of analysis protocols related to the ISHAGE, although some deviations from the originally recommended protocol have been highlighted [25]. The higher frequency of samples with very low absolute CD34+ cell levels during the 2014–2024 decade may explain the presence of some cases with an unexpectedly higher %aTE and the slight positive trend of the regression line.

Dual platform analysis seems more or less able to be on target with the expected central result values of exercises using stabilized PB, although with wider CVs [25] and higher %aTE. Conversely, DP is in trouble with real patient samples, both on the FCM and the automated blood counter sides, it is highly inaccurate with fresh concentrated apheresis and cord blood, and cannot be applied at all with thawed samples [21]. Scientific evidence should put pressure on the residual DP users to prompt an universal change to the far more reliable SP technique.

An excess variability of the measurement of the absolute level of CD34+ cells may have clinical consequences. For instance, the misclassification of patients with hematological disorders and the under- or over-estimation of the critical checkpoints of the CD34+ HPC transplantation procedure. The CD34+ PB trigger values for starting the HPC apheresis are strong predictors of the overall collection [37], as well as the post-apheresis PB level that are used to calculate the cell yield [38]. The worst error in this context is the overestimation of absolute CD34+ HPC levels that may trigger

long and inefficient collections, with the need of additional procedures, or the reinfusion of too few viable CD34+ HPCs with the thawed apheresis products. As mentioned, the absolute CD34+ levels in the concentrated apheresis suspension are still not evaluable with EQA schemes.

An attempt should be made to translate our findings into daily practice. Commercially available reference materials for internal quality control are useful and mandatory for the clinical laboratory, but they invariably come with just an approximate target level of CD34+ cells/μL and a very wide acceptable range of results [39]. This makes such preparations not very useful to assess the desirable or optimal imprecision levels of the CD34 assay. As a matter of fact, a too wide distribution of the expected results does not provide the necessary indications to strive for goals of minimized assay imprecision. As such the improvement of existing materials by commercial providers to develop a widely available, stable control material with a clinically relevant acceptable range is crucial for laboratories to maintain confidence in their results. Performing replicate measurements of the stabilized UKNEQAS samples shortly after the vial opening, thanks to their high stability, the low intrinsic variability and the known consensus results from exercise reports, are useful to refine laboratory procedures, to reach SOTA-desirable and possibly optimal counting performance.

It seems however likely that the tight %aTE values obtained with stabilized samples would not be achievable with real-world patient samples, due to the variable underlying pathological conditions, the individually unpredictable response to mobilizers, the high number of interfering accompanying cells and the CD34 antigen modulation induced by apheresis. Optimizing the analysis procedures as discussed is however a fundamental prerequisite to improve patients' diagnoses and transplant outcomes.

A major limitation of our study is the lack of a parallel analysis with concentrated leukapheresis preparations, that entail a number of delicate preparatory and preanalytical steps and are characterized by a much larger chance of generating abnormal results with unfavorable clinical impact. A similar study of %aTE in apheresis products, cord blood and thawed apheresis samples seems not yet possible at present, given that these materials are hardly available for distribution in large-scale EQA/PT programs.

Some large studies have been already published on BV of lymphocyte subsets [40, 41] and tables reporting BV parameters for normal subpopulations levels are publicly available. However, BV parameters for absolute CD34+ cell levels in PB may be generated at present for healthy steady-state subjects only (i.e., <10 cells/μL), thanks to large cooperative studies. The complex dynamics of absolute CD34+ cell levels in mobilized subjects and in the transplantation procedures seem at present hardly compatible with the generation of reliable BV tables, as for other ordinary analytes.

To conclude, the evaluation of the total allowable imprecision of CD34+ cell levels and other FCM analyses providing quantitative results of clinical relevance is now an issue of the utmost importance. Laboratorians should be fully aware of the current performance status of their measurement processes and of the improvement goals that have to be achieved with time and practice.

The analysis of %aTE of absolute CD34+ cell levels is still in its infancy. Since %aTE is a clinical parameter influenced by technology, practice and geography, scientific societies with the help of EQA/PT scheme providers should be able to establish and recommend the desirable and optimal aTE% levels in all the clinical settings, taking into account the necessary continuous improvement of this parameter with time and technical progress.

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References

1. Jones GRD. Using analytical performance specifications in a medical laboratory. Clin Chem Lab Med 2024;62:1512–19.
2. ISO 15189:2022 medical laboratories international standard — requirements for quality and competence. https://www.iso.org/obp/ui/#iso:std:iso:15189:ed-4:v1:en.

3. ISO/TS 20914:2019 Medical laboratories — practical guidance for the estimation of measurement uncertainty. https://www.iso.org/obp/ui/en/#iso:std:iso:ts:20914:ed-1:v1:en.
4. Braga F, Panteghini M. Generation of data on within-subject biological variation in laboratory medicine: an update. Crit Rev Clin Lab Sci 2016; 53:313–25.
5. Johnson PR, Shahangian S, Astles JR. Managing biological variation data: modern approaches for study design and clinical application. Crit Rev Clin Lab Sci 2021;58:493–512.
6. Sciacovelli L, Padoan A, Aita A, Basso D, Plebani M. Quality indicators in laboratory medicine: state-of-the-art, quality specifications and future strategies. Clin Chem Lab Med 2023;61:688–95.
7. Sandberg S, Coskun A, Carobene A, Fernandez-Calle P, Diaz-Garzon J, Bartlett WA, et al. Analytical performance specifications based on biological variation data - considerations, strengths and limitations. Clin Chem Lab Med 2024;62:1483–9.
8. Westgard QC. CLIA acceptance limits for proficiency testing; 2024. https://westgard.com/clia-a-quality/quality-requirements/2024-clia-requirements.html [Accessed Jan 2025].
9. Data innovation - total allowable error table (Updated June 2024). https://www.datainnovations.com/allowable-total-error-table/ [Accessed Jan 2025].
10. Aarsand AK, Fernandez-Calle P, Webster C, Coskun A, Gonzales-Lao E, Diaz-Garzon J, et al. European federation of clinical chemistry and laboratoy medicine (EFLM). Biological Variation Database. https://biologicalvariation.eu [Accessed Jan 2025].
11. Sun diagnostics - recommended total allowable error limits; 2024. http://rmbiolab.com/UpFiles/Documents/808edaad-0fb5-4412-9235-2921bf9e8b62.pdf. [Accessed Jan 2025].
12. Fraser CG, Hyltoft Petersen P, Libeer JC, Ricos C. Proposals for setting generally applicable quality goals solely based on biology. Ann Clin Biochem 1997;34:8–12.
13. Fraser CG. Reference change values. Clin Chem Lab Med 2012;50: 807–12.
14. Eidenschink L, Di Zerega G, Rodgers K, Bartlett M, Wells DA, Loken MR. Basal levels of CD34 positive cells in peripheral blood differ between individuals and are stable for 18 months. Cytom B Clin Cytom 2012;82: 18–25.
15. Jelic TM, Estalilla OC, Vos JA, Harvey G, Stricker CJ, Adelanwa AO, et al. Flow cytometric enumeration of peripheral blood CD34$^+$cells predicts bone marrow pathology in patients with less than 1% blasts by manual count. J Blood Med 2023;14:519–35.
16. Gratama JW, Orfao A, Barnett D, Brando B, Huber A, Janossy G, et al. Flow cytometric enumeration of CD34+ hematopoietic stem and progenitor cells. European Working Group on Clinical Cell Analysis. Cytometry 1998;34:128–42.
17. Dauber K, Becker D, Odendahl M, Seifried E, Bonig H, Tonn T. Enumeration of viable CD34(+) cells by flow cytometry in blood, bone marrow and cord blood: results of a study of the novel BD™ stem cell enumeration kit. Cytotherapy 2011;13:449–58.
18. Castelhano MV, Reis-Alves SC, Vigorito AC, Rocha FF, Pereira-Cunha FG, De Souza CA, et al. Quantifying loss of CD34+ cells collected by apheresis after processing for freezing and post-thaw. Transfus Apher Sci 2013;48:241–6.
19. Teipel R, Oelschlägel U, Wetzko K, Schmiedgen M, Kramer M, Rücker-Braun E, et al. Differences in cellular composition of peripheral blood stem cell grafts from healthy stem cell donors mobilized with either granulocyte colony-stimulating factor (G-CSF) alone or G-CSF and plerixafor. Biol Blood Marrow Transpl 2018;24:2171–7.
20. Fournier D, Lewin A, Simard C, Trépanier P, Néron S, Ballerini L, et al. Multi-laboratory assay for harmonization of enumeration of viable CD34+ and CD45+ cells in frozen cord blood units. Cytotherapy 2020; 22:44–51.
21. CLSI. CD34+ hematopoietic stem cell enumeration by flow cytometry, 1st ed. CLSI guideline H63. Wayne, PA, U.S.A.: Clinical and Laboratory Standards Institute, in preparation. https://clsi.org/standards-development/projects-in-progress/.
22. Fernández-Luis S, Comins-Boo A, Pérez-Pla F, Irure Ventura J, Insunza Gaminde A, López-Hoyos M, et al. Allowable total error in CD34 cell analysis by flow cytometry based on State of the Art using Spanish EQAS data. Clin Chem Lab Med 2024;63:367–75.
23. Osborne JW, Overbay A. The power of outliers (and why researchers should ALWAYS check for them). Practical Assess Res Eval 2004;9:6.
24. Rousseew PJ, Hubert M. Anomaly detection using robust statistics. WIREs Data Mining Knowl Discov 2018;8:e1236.
25. Whitby A, Whitby L, Fletcher M, Reilly JT, Sutherland DR, Keeney M, et al. ISHAGE protocol: are we doing it correctly? Cytom B Clin Cytom 2012; 82:9–17.
26. Cesana C, Klersy C, Brando B, Nosari A, Scarpati B, Scampini L, et al. Prognostic value of circulating CD34+ cells in myelodysplastic syndromes. Leuk Res 2008;32:1715–23.
27. Ogata K, Della Porta MG, Malcovati L, Picone C, Yokose N, Matsuda A, et al. Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: a prospective validation study. Haematologica 2009;94: 1066–74.
28. Barosi G, Campanelli R, Catarsi P, Abbà C, Carolei A, Massa M, et al. Type 1 CALR mutation allele frequency correlates with CD34/CXCR4 expression in myelofibrosis-type megakaryocyte dysplasia: a mechanism of disease progression? Blood Cancer J 2024;14:18.
29. Giralt S, Costa L, Schriber J, DiPersio J, Maziarz R, McCarty J, et al. Optimizing autologous stem cell mobilization strategies to improve patient outcomes: consensus guidelines and recommendations. Biol Blood Marrow Transpl 2014;20:295–308.
30. Duong HK, Savani BN, Copelan E, Devine S, Costa LJ, Wingard JR, et al. Peripheral blood progenitor cell mobilization for autologous and allogeneic hematopoietic cell transplantation: guidelines from the American Society for Blood and Marrow Transplantation. Biol Blood Marrow Transpl 2014;20:1262–73.
31. Nabity MB, Harr KE, Camus MS, Flatland B, Vap LM. ASVCP guidelines: allowable total error hematology. Vet Clin Pathol 2018;47:9–21.
32. Iurlo A, Galli N, Bucelli C, Artuso S, Consonni D, Cattaneo D. Trend of circulating CD34+ cells in patients with myelofibrosis: association with spleen response during ruxolitinib treatment. Br J Haematol 2023;200: 315–22.
33. Luque-Paz D, Cottin L, Lippert E, Robin JB, Bescond C, Genevieve F, et al. Different number of circulating CD34 + cells in essential thrombocythemia, prefibrotic/early primary myelofibrosis, and overt primary myelofibrosis. Ann Hematol 2022;101:893–6.
34. Mannelli F, Bencini S, Coltro G, Loscocco GG, Peruzzi B, Rotunno G, et al. Integration of multiparameter flow cytometry score improves prognostic stratification provided by standard models in primary myelofibrosis. Am J Hematol 2022;97:846–55.
35. Brando B, Gatti A. CD34+ progenitor cells meet metrology. Clin Chem Lab Med 2024;63:225–7.
36. Thompson M. The amazing Horwitz function. AMC Technical Brief No.17 July 2004, Royal Society of Chemistry. https://www.rsc.org/images/horwitz-function-technical-brief-17_tcm18-214859.pdf [Accessed Jan 2025].

37. Jacob RP, Walsh EM, Maslak PG, Giralt SA, Avecilla ST. A simplified CD34+ based preharvest prediction tool for HPC(A) collection. Transfusion 2021;61:1525–32.
38. Ford CD, Greenwood J, Strupp A, Lehman CM. Change in CD34+ cell concentration during peripheral blood progenitor cell collection: effects on collection efficiency and efficacy. Transfusion 2002;42: 904–11.
39. Ferrero I, Rustichelli D, Castiglia S, Gammaitoni L, Polo A, Pautasso M, et al. Inter-laboratory method validation of CD34+ flow-cytometry assay: the experience of Turin Metropolitan Transplant Centre. EJIFCC 2023;34:220–7.
40. Li C, Wang Y, Lu H, Du Z, Xu C, Peng M. Study of total error specifications of lymphocyte subsets enumeration using China National EQAS data and Biological Variation Data Critical Appraisal Checklist (BIVAC)-compliant publications. Clin Chem Lab Med 2020;59:179–86.
41. Comins-Boo A, Pérez-Pla F, Irure-Ventura J, López-Hoyos M, Blanco-Peris L, del Carmen Martín Alonso M, et al. Total error in lymphocyte subpopulations by flow cytometry-based in state of the art using Spanish EQAS data. Clin Chem Lab Med 2023;62:312–21.

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