

Use of allogeneic apheresis stem cell products as an interlaboratory proficiency challenge

Laura Cooling,¹ Kelly Roxbury,¹ Sandra Hoffmann,¹ Joan DeBusscher,¹ Usha Kota,¹ Steven Goldstein,^{2,3} and Robertson Davenport¹

BACKGROUND: AABB Standards requires that laboratories participate in a proficiency test (PT) program for critical analytes. Institutions can purchase commercial PT materials; however, PT can also be performed through interlaboratory exchange. We investigated the utility of allogeneic hematopoietic progenitor cell apheresis (HPC-A) products as an interlaboratory PT challenge for total nucleated cell count (TNC) and CD34 assessment.

STUDY DESIGN AND METHODS: Three-year retrospective and comparative review of unrelated allogeneic HPC-A products received by the University of Michigan between January 2011 and December 2013. Internal TNC and CD34 count were compared to the external collecting facility by paired t test and linear regression. The absolute and percent difference between external and internal counts and 95% limits of agreeability (95% LA) were determined. Results were analyzed relative to donor center location (international, domestic), time zone (domestic), and calendar year.

RESULTS: There was a strong correlation between internal and external TNC, regardless of donor center location or year. For CD34, there was a good correlation between centers ($R = 0.88-0.91$; slope = $0.95-0.98x$) with a median difference of -1% (95% LA, -50% , $+47\%$). This was considerably better than commercial PT challenges, which showed a persistent negative bias for absolute CD34 and CD3 counts.

CONCLUSION: Allogeneic HPC-A products represent an interlaboratory PT exchange for all critical analytes, including TNC and CD34 count, cell viability, and sterility. Allogeneic HPC-A products, which are fresh and transported under validated conditions, are less subject to preanalytical variables that may impact commercial PT samples such as aliquoting and sample homogeneity, commercial additives, and sample stability during manufacturing and transport.

Both AABB and the Foundation for the Accreditation of Cell Therapy (FACT) require assessment and documentation of laboratory proficiency in cell therapy processes.^{1,2} FACT Standard D8.1.4 requires a process for monitoring reliability, accuracy, precision, and performance of laboratory test procedures, including documentation of ongoing proficiency testing (PT).² AABB Standards for Cell Therapy Services are more specific, mandating participation in a PT program for each measured analyte (8.2).¹ Moreover, US-based laboratories are required to participate in a Centers for Medicare & Medicaid-approved PT program for each Clinical

ABBREVIATIONS: 7-AAD = 7-aminoactinomycin D; CAP-SCP = College of American Pathology–stem cell processing; CoA = certificate of analysis; FACT = Foundation for the Accreditation of Cell Therapy; HPC-A = hematopoietic progenitor cell apheresis; ISHAGE = International Society for Hematotherapy and Graft Engineering; 95% LA = 95% limits of agreeability; LMD = list mode data; NMDP = National Marrow Donor Program; PT = proficiency test or testing; SS = side scatter; TNC = total nucleated cell count; UK NEQA = United Kingdom National External Quality Assessment.

From the ¹Department of Pathology and the ²Department of Internal Medicine, Division of Hematology/Oncology and Blood and Marrow Transplant Program, University of Michigan, Ann Arbor, Michigan; and the ³Bone Marrow Transplant Program, Florida Hospital Cancer Institute, Orlando, Florida.

Address reprint requests to: Laura Cooling, MD, MS, University of Michigan Hospitals and Clinics, 2F225 UH-Blood Bank, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-0054; e-mail: lcooling@med.umich.edu.

Received for publication December 8, 2016; revision received February 2, 2017; and accepted February 7, 2017.

doi:10.1111/trf.14107

© 2017 AABB

TRANSFUSION 2017;57;1543–1554

Laboratory Improvement Amendments–regulated analyte. For other analytes, there must be a system for deeming the accuracy and reliability of test results (8.2.1).¹ In cell therapy, critical analytes include the total nucleated cell count (TNC), CD34 count, CD34 viability, and sterility testing, which are required for all cell therapy products per AABB Standard 5.17A and FACT Standards D8.1.3 and D8.7.^{1,2} In the United States, TNC, CD34, and cell viability are nongraded PT analytes, but are regulated and must be assessed twice yearly to determine the accuracy and reliability of the reported results.

We have participated in the biannual College of American Pathology's (CAP) stem cell processing (SCP) PT challenges for several years. Each CAP-SCP challenge includes two prepared samples resuspended in commercial tissue culture medium supplemented with 10% human sera.³⁻¹⁰ Participants are asked to measure the TNC, CD34 count, and cell viability per institutional procedures. The reported results are analyzed relative to instrumentation, reagents, and CD34 testing platform (single- or dual-stage). Due to the small number of participants and wide variation in testing results, the CAP-SCP PT challenges are currently ungraded.

Another avenue for PT is interlaboratory exchanges. This method is not uncommon for new molecular testing assays or infrequently performed assays.¹¹ In this respect, allogeneic stem cell products procured through the National Marrow Donor Program (NMDP) can be considered an interlaboratory PT challenge, since these products are tested by both collection and receiving facilities. Unlike commercial PT samples, allogeneic products are fresh and are not subject to dilution or modification by the addition of stabilizing agents or resuspension in tissue culture medium.^{3-10,12-18} To assess the utility of allogeneic products for PT, we compared the TNC and CD34 count from 141 peripheral blood hematopoietic progenitor cell apheresis (HPC-A) units received at our institution from external collection facilities over a 3-year period.

MATERIALS AND METHODS

Study design

The study was a 3-year retrospective review of all unrelated allogeneic HPC-A units received by the University of Michigan between January 2011 and December 2013. External and internal testing results for the calculated absolute TNC and CD34 count were compared and analyzed as an interlaboratory PT challenge. Data from the external donor facility included the absolute TNC ($\times 10^9$) and CD34 count ($\times 10^6$) of each unit, product volume (mL), whether it was collected by a NMDP or non-NMDP donor facility, NMDP donor center identification number, donor center location (country, state), donor sex, and age. Donor centers were classified as international, if located

outside the United States, or domestic, if located within the continental United States. For domestic donor facilities, the time zone (Eastern, Central, Mountain, Pacific) was also included for analysis. Available internal laboratory data included the absolute TNC and CD34 count, cell viability, percent mononuclear cells (MNCs), hematocrit (Hct; %), platelet (PLT) count ($\times 10^9/L$), and transplant cell dose.

Internal cell analysis

Allogeneic products were tested for TNC, CD34 count, cell viability, and sterility testing upon receipt. TNC and complete blood count were performed using an automated cell counter (Sysmex XE-5000, Kobe). The white blood cell (WBC) differential was determined by manual methods. Per protocol, samples with an initial WBC count of more than $3 \times 10^{11}/L$ were diluted 1:5 in commercial cell diluent (Cell Pack DCL, Sysmex) and reanalyzed.

For CD34 analysis, a 0.5-mL aliquot was incubated with a cocktail-containing antibodies against CD34, CD45, CD14, and 7-aminoactinomycin D (7-AAD) for 10 minutes, followed by red blood cell lysis and immediate analysis: Samples were not subjected to a wash step or fixation before flow cytometry. CD34 analysis was performed using a dual-stage, four-color modified International Society for Hematotherapy and Graft Engineering (ISHAGE) protocol on a Gallios multichannel flow cytometer (Beckman Coulter). Per ISHAGE, CD34 cells were identified through sequential gating using CD45, CD34, and forward and side scatter (SS) to identify CD34+, CD45^{dim}, SS^{low} HPC cells.^{17,19} In addition, samples were costained with an anti-CD14 to exclude CD34+, CD14+ cells.^{14,20-22} Cell viability was determined with 7-AAD (Life Technologies). A minimum of 100,000 total events and/or 2000 CD34 cell events were acquired per analysis. Anti-CD34 (clone 581, Class III; phycoerythrin [PE] conjugate), anti-CD45 (Clone J33, electron-coupled dye conjugate), anti-CD14 (Clone RM052, fluorescein isothiocyanate [FITC] conjugate) were purchased from Beckman Coulter. Ammonium chloride lysing reagent was prepared fresh daily from stock reagents. Daily quality controls (QCs) for flow cytometry included commercial reagents for CD34 and CD45 (Chex CD-Plus BC and CD-Chex CD34, Streck).

Statistical analysis

The TNC and CD34 count for individual units as measured by the donor center (external) and our institution (internal) were compared by paired t test (Fig. 1) and linear regression. In addition, the absolute difference between donor center and internal cell counts (external count – internal count) and the percent (%) difference (external count – internal count/external count) were also calculated. Results were reported as the mean \pm standard deviation (SD). The 95% limits of agreeability (95% LA) were

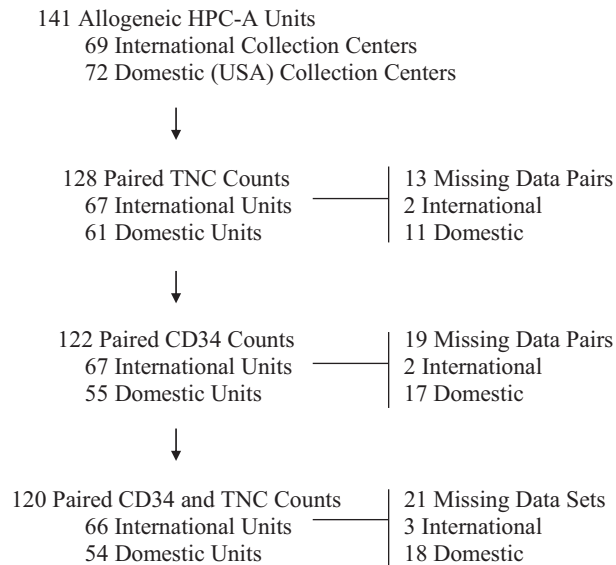


Fig. 1. Paired data sets by donor center location, CD34 count, and TNC.

calculated and plotted as described.^{23,24} Specifically, the percent difference was plotted against the mean absolute CD34 count ($[\text{external count} + \text{internal count}] \div 2$). Results falling between the mean $\pm 1.96\text{SD}$ were considered within the 95% LA. Differences between international and domestic HPC-A products were compared by standard t test. Linear regression, graphing, and t tests were performed using computer software (Kaleidograph, Synergy Software). Categorical variables were compared by chi square using computer software (Epi Info, Centers for Disease Control and Prevention).

CAP-SCP PT analysis

CAP-SCP PT samples (2.5-mL sample) were shipped overnight with cold gel packs. Upon arrival, samples were mixed and sterily split into four 0.5-mL aliquots in a biological hood for hematology, flow cytometry, Gram stain, and bacterial culture. With one exception, all samples were tested and analyzed on the day of receipt per institutional protocols as described. All samples were tested within 24 hours of receipt per CAP requirements.

TNC and WBC count were performed on a cell counter (XE-5000, Sysmex). CD34 analysis was performed using a modified ISHAGE protocol as described and reported as %CD34 and CD34 count ($\times 10^6$). In addition, the list mode data (LMD) files were reanalyzed at a later date by a second individual, who was blinded with regard to the original testing results. The %CD34 was determined without 7-AAD gating as recommended by the United Kingdom National External Quality Assessment program (UK NEQA).¹⁷

For analysis, our internal institutional CAP-SCP results for TNC and CD34 results were plotted against the

TABLE 1. External donor center demographics

Collection centers	Number of centers	Number of units
All donor centers	40	141
International	17	69
NMDP	7	58
Non-NMDP	10	11
Number of countries	9	
Europe	14	66
Mideast	2	2
Australia	1	1
Domestic	23	72
NMDP	23	72
Non-NMDP	0	0
Number of states	15	
Eastern*	8	39
Central*	4	14
Mountain*	2	12
Pacific*	1	7

*Time zone of external donor center.

manufacturer's certificate of analysis (CoA) as provided by CAP.³⁻¹⁰ To compare our results with other participants, the mean $\pm 1\text{SD}$ and $\pm 2\text{SD}$ for all peer institutions were plotted in parallel. For CD34, our results were compared only to participants using a dual-stage platform for CD34 enumeration. As a control, we performed the same analysis for the %CD3 and absolute CD3 count ($\times 10^6$) results, which were also included in the CAP-SCP challenges. Internal results were compared to CoA and participant mean by paired t test. In addition, the absolute and percent difference in TNC, CD34, and CD3 results were calculated and compared.

RESULTS

External allogeneic HPC-A units

A total of 141 units HPC-A units for 131 patients were received from 40 external collection centers between 2011 and 2013 (Table 1). The vast majority of units (130, 92%) were collected at NMDP-affiliated centers. Sixty-nine units were from international collection centers and 72 units were from domestic centers located in the United States. Domestic units were collected from 23 NMDP centers located in 15 states. Half of all domestic units (39/72, 54%) were collected at centers located in the same time zone (Eastern) as our facility.

Most international units were from European donor centers. Germany was the largest international supplier of HPC-A units (55, 83%), with nearly 75% of all international units (49/66) coming from a single collection center. A limited number of units (1-2 units) were received from eight other countries including Poland (2), Denmark (1), England (3), Portugal (2), Sweden (2), Netherlands (1), Israel (2), and Australia (1). All non-NMDP units were from European collection centers. HPC-A units from domestic and international centers were comparable with

TABLE 2. Comparison of allogeneic HPC-A units by donor center location*

Variable	International	Domestic	p value
Number of donors	65	66	
Sex (male/female)	55/10	49/17	0.15
Age (years)	33.1 ± 9.3	34.5 ± 10.3	0.40
Total number of units	69	72	
Number of split units	4	6	
Unit volume (mL)	360 ± 108	348 ± 109	0.53
TNC (×10 ⁹)*	79.35 ± 27.56	83.78 ± 38.92	0.44
Cell viability (%)*	93.7 ± 11.8	96.4 ± 12.0	0.17
% MNCs*	68.0 ± 20.0	67.6 ± 20.2	0.90
Total MNCs (×10 ⁹)*	50.15 ± 14.18	52.37 ± 19.49	0.44
MNCs/kg (×10 ⁸)*	6.35 ± 3.00	7.18 ± 5.37	0.26
%CD34*	0.83 ± 0.36	0.87 ± 0.51	0.88
Total CD34 (×10 ⁶)*	638.65 ± 272.42	696.71 ± 357.75	0.31
CD34/kg (×10 ⁶)*	8.41 ± 5.58	9.01 ± 8.30	0.61
Hematocrit (%)*	3.9 ± 1.8	4.5 ± 2.4	0.14
PLTs (×10 ⁹ /L)*	2154 ± 959	2525 ± 1018	0.03

*Based on internal cell counts.

few differences relative to donor characteristics, volume, total cell counts, and cell dose (Table 2).

Comparison of TNC

External and internal TNC were available in 128 units (Fig. 1). Paired counts were initially compared by linear regression (Fig. 2A), which showed a strong correlation ($R = 0.93$) with a slope (m) of nearly 1 ($m = 0.86$). The same tight correlation was observed for both international ($R = 0.92$) and domestic units ($R = 0.93$). The y-intercept (17.3) indicated a trend toward higher internal TNC, especially for domestic units.

The absolute and percent difference in TNC were also determined and compared (Figs. 2B and 2C). Overall, the TNC in 84% (108/128) of units were within $\pm 10\%$ of each other (Fig. 2C). For domestic units, there was a slight bias toward higher internal counts (64% of units), although the median percent difference was modest (-3.3% , Table 3). There was a trend ($p = 0.07$) toward higher internal TNC for HPC-A units collected by centers located in the Eastern time zone.

External counts for international units were, in general, remarkably close to internal counts (Table 3). The mean and median percent differences were 1.35 and 5.1%, respectively. We also examined TNC by continent and donor center since 75% of all international units came from a single donor center (107). As shown in Table 3, external TNC from donor center 107 tended to be 5% higher than internal counts ($p = 0.002$).

Comparison of CD34 counts

Paired external and internal CD34 counts were available in 122 (86.5%) units (Fig. 1). Like TNC, there was a close correlation between external and internal CD34 counts (Fig. 3A). As shown in Table 3, there was no significant

difference in mean CD34 counts between external donor facilities and internal testing ($p = 0.73$), with a median percent difference of -1% (95% LA, -50% , $+47\%$; Fig. 3C). Units that exceeded 95% LA tended to have lower CD34 counts. Overall, the internal CD34 count was within $\pm 10\%$ of the collecting facility's yield in 72 units (59%) and within 20% in 94 units (77%).

When examined by donor center location, 41 of 55 (74.5%) domestic units were within $\pm 10\%$, with a slight bias toward higher internal CD34 counts at our facility (median % difference, -0.8% ; 95% LA, -44.7 , 43.1). The median percent difference in CD34 counts for international units was -0.7% (Table 3; 95% LA, -45.5 , 44.1), with nearly half falling within $\pm 10\%$.

TNC and CD34 results by year

We also compared TNC and CD34 counts by calendar year (Fig. 4). There was a small improvement in TNC correlation (Fig. 4A) between years 2011 ($R = 0.84$, $m = 0.6x$) versus 2012 and 2013 ($R = 0.96-0.98$, $m = 0.93x-1x$). There was no significant difference in TNC by paired t test ($p = 0.23-0.81$, Table 3). There was also no significant difference in the relative distribution of international (43%-52%) versus domestic HPC-A units over the 3-year period.

There was a good correlation between CD34 counts over time (Fig. 4B; $R = 0.88-0.90$). A comparison of counts by paired t test showed no significant differences although there was a trend toward higher external counts in 2013 ($p = 0.07$; Table 1, 4.6%). The higher external counts may reflect a 10% increase in the number of domestic HPC-A units collected by centers located within the Eastern time zone (36% in 2013).

Outlier analysis

A detailed analysis was performed in 10 cases in which the percent difference in either TNC or CD34 count was

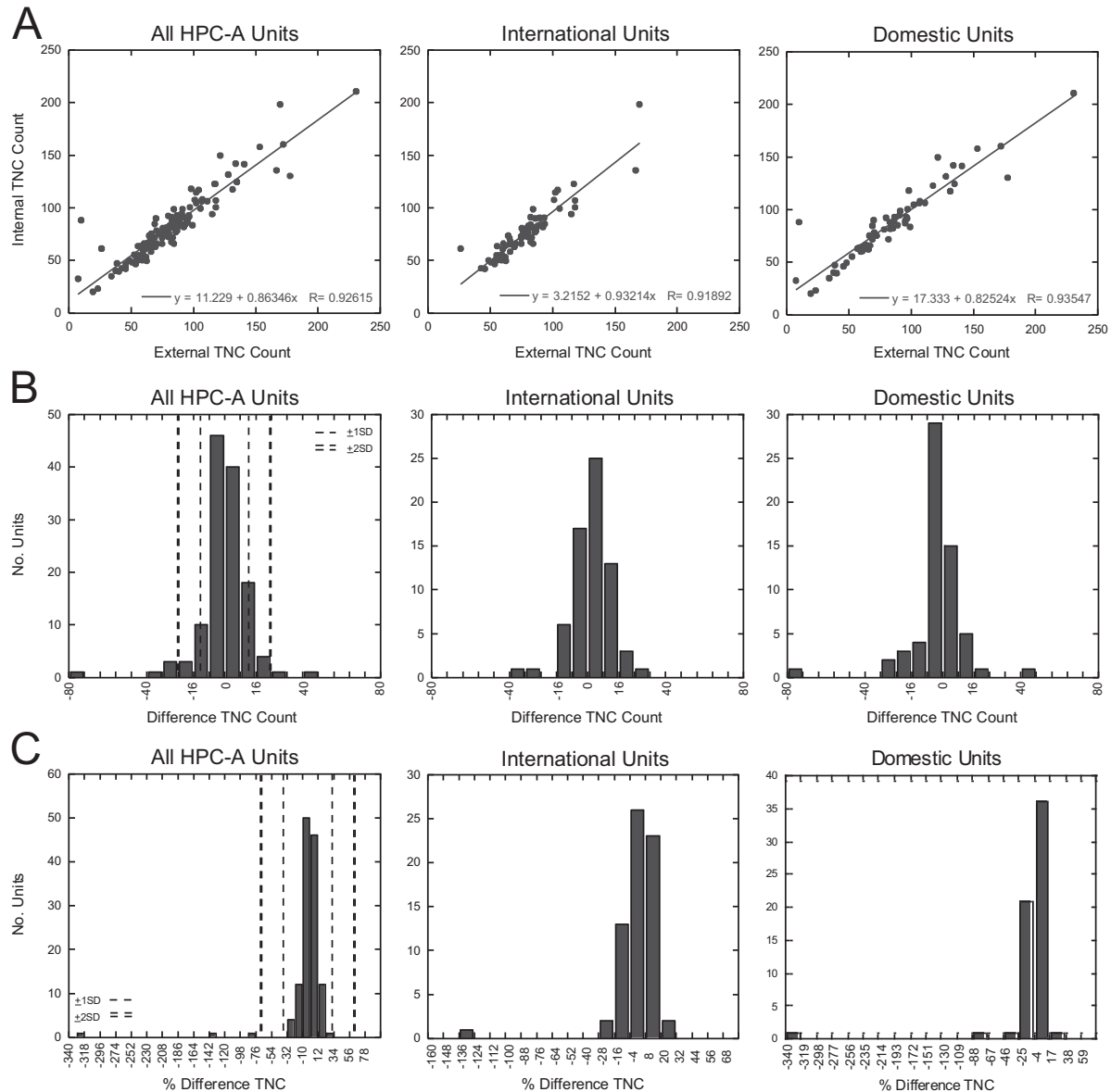


Fig. 2. Comparison of TNC in allogeneic HPC-A products by donor center location. (A) Correlation between internal and external TNC ($\times 10^8$). (B) Absolute difference in TNC (external - internal). (C) Percent (%) difference in TNC. Vertical lines indicate $\pm 1SD$ (- -) and $\pm 2SD$ (= =).

more than 50% (Table S1 [available as supporting information in the online version of this paper], bold). Outlier counts were observed with both international ($n = 5$) and domestic ($n = 5$) units. A majority of units (6/10) were collected during the 2011 calendar year (6/33, 18%) versus three (6%) in 2012 and only one (2%) in 2013. All six cases in 2011 demonstrated either higher internal TNC (70%-382%) or higher CD34 counts (63%-94%). Four samples showed decreases in both TNC and CD34 count, as well as lower cell viability (88%-94%) suggesting some product deterioration during transit.

Commercial PT performance

We participate in a commercial stem cell proficiency challenge offered biannually by CAP. We compared our results for TNC, CD34, and CD3 from 16 PT samples against the expected results based on the manufacturer's CoA and the mean result ($\pm 1SD$ and $\pm 2SD$) for peer participants.

In general, our results for TNC fell within $\pm 2SD$ for all challenges (Fig. 5A). In paired t tests, our results were -1.8% lower than the CoA ($p = 0.027$; range, -23.4% to $+15.3\%$) but 4.9% higher than the participant mean ($p = 0.07$; range, -9% , 6.8%). The mean coefficient of variation (CV) across all TNC challenges was $9.2\% \pm 2.3\%$.

TABLE 3. Paired comparison of internal and external TNC and CD34 count*

	Number of units	Total cell counts		p value§	External – internal	
		External†	Internal‡		[Difference] (median)	% difference¶ (median)
TNC (×10⁹)						
All units	128	82.06 ± 33.98	82.22 ± 31.82	0.88	-0.2 (-0.2)	-3.7 (-0.15)
2011	32	78.90 ± 35.46	79.61 ± 27.24	0.57	-20.0 (0.2)	-14.9 (0.1)
2012	50	85.78 ± 31.88	87.47 ± 38.08	0.81	-0.3 (-1.0)	-0.6 (-1.4)
2013	46	80.31 ± 34.43	76.88 ± 32.80	0.23	1.3 (1.5)	0.97 (2.0)
International	67	78.67 ± 24.64	76.49 ± 25.08	0.87	2.1 (3.3)	1.35 (5.1)
Europe	64	78.00 ± 22.15	76.22 ± 24.08	0.14	1.8 (3.4)	1.89 (5.2)
NMDP #107	49	79.50 ± 17.98	76.05 ± 18.24	0.002	2.7 (3.4)	4.29 (5.4)
Other	15	76.77 ± 32.57	76.77 ± 58.03	0.28	-1.6 (0.9)	-8.96 (-2.1)
Mideast	2	104.5 ± 87.5	89.19 ± 65.8	0.50	15.3 (15.3)	9.2 (9.2)
Australia	1	65.88	68.62		-2.74	-4.1
Domestic	61	84.52 ± 40.64	87.08 ± 35.85	0.17	-2.6 (-2.4)	-9.3 (-3.3)
Eastern	35	78.08 ± 35.17	83.96 ± 30.51	0.07	-4.9 (-2.8)	-15.1 (-3.6)
Central	10	89.65 ± 45.24	81.45 ± 33.86	0.24	6.2 (0.9)	2.5 (-2.3)
Mountain	12	81.45 ± 32.77	84.11 ± 32.71	0.12	-2.6 (-1.0)	-3.6 (-1.7)
Pacific	4	142.23 ± 62.67	146.16 ± 52.77	0.71	-3.9 (-3.4)	-4.9 (-2.3)
Total CD34 (×10⁶)						
All units	122	665.67 ± 315	669.25 ± 341	0.80	-4.9 (-5.5)	-3.1 (-1.1)
2011	30	687.39 ± 311	704.17 ± 381	0.61	-16.8 (-20.7)	-11.4 (-9.8)
2012	48	691.37 ± 311	724.58 ± 366	0.15	-21.2 (-9.8)	-6.8 (-2.5)
2013	44	613.12 ± 253	578.71 ± 261	0.07	34.4 (19.8)	4.6 (3.2)
International	67	640.18 ± 274	655.0 ± 286	0.29	-12.2 (4.1)	-5.0 (-0.7)
Europe	64	630.45 ± 269	649.46 ± 285	0.21	-15.8 (0.6)	-5.3 (-0.8)
NMDP #107	48	676.14 ± 278	696.4 ± 98	0.21	-16.1 (5.3)	-4.6 (-0.4)
Other	16	493.14 ± 186	508.5 ± 186	0.68	-15.3 (-9.5)	-7.3 (-1.3)
Mideast	2	714.5 ± 401	650.9 ± 365	0.24	63.6 (63.6)	0 (0)**
Australia	1	1114	1043		-71	-6.4%
Domestic	55	696.71 ± 358	686.15 ± 400	0.68	-16.08 (-3.1)	-0.8 (-0.8)
Eastern	34	684.69 ± 355	688.38 ± 398	0.91	-5.22 (4.7)	-1.8 (-2.0)
Central	9	564.78 ± 267	564.78 ± 221	0.61	26.4 (33)	-3.0 (-5.1)
Mountain	10	809.44 ± 459	799.47 ± 548	0.88	9.97 (-49.6)	-0.3 (-10.7)
Pacific	2	812.2 ± 108	627.7 ± 216	0.25	184.5 (184.5)	23.8 (23.8)

*Limited to HPC-A products with both external and internal testing results (see Fig. 1).

†Absolute counts from external donor facility, reported as mean ± SD.

‡Internal absolute counts, reported as mean ± SD.

§Paired t test.

||Mean (median) difference in absolute counts between external donor center and internal results, where external – internal count.

¶Mean (median) percent (%) difference in absolute counts between external donor center and internal results, where [(external – internal count) ÷ 2].

**Percent difference was -8.9% and +8.9% (n = 2).

There was no CoA for %CD34, limiting our analysis to peer participants. As shown in Fig. 5B, internal results for %CD34 were within ±1SD for dual-stage users (p = 0.48; CV range, 9.3%-46.9%). In contrast, the absolute CD34 count (×10⁶) was consistently low (-2SD, Fig. 5C) relative to the CoA (P = 0.007) and participant mean (p = 0.002; CV range, 14%-59.4%). Based on the recommendations of the UK NEQA program for HPC analysis, we reanalyzed the LMD file from each challenge keeping the 7-AAD gate open.¹⁷ There was no significant change in CD34 results (data not shown). We also examined whether there was sufficient sample to collect the minimum number of cell events as recommended by ISHAGE (>75 × 10³ CD45+ cells, >100 CD45+, CD34+ cells).¹⁹ Although sufficient CD45+ events were collected, the minimum number of CD34+ events could not be reached in three samples (range, 41-67 CD34 cells).

Because of our consistently low absolute CD34 counts, we also examined our performance with %CD3 and absolute CD3 (×10⁶) counts during the same challenges. Unlike CD34+ cells, CD3+ cells are plentiful and account for 55% to 84% of all peripheral blood lymphocytes.²⁵ As shown in Figs. 5D and 5E, the %CD3 (p = 0.0005) and absolute CD3 counts (p = 0.0001) were significantly lower than the participant mean. The lower TNC, %CD3, and absolute CD3 counts suggest some sample deterioration before receipt and testing.

DISCUSSION

The TNC and viable CD34 count are critical to clinical decision making during stem cell collection and transplantation. Numerous clinical studies over two decades

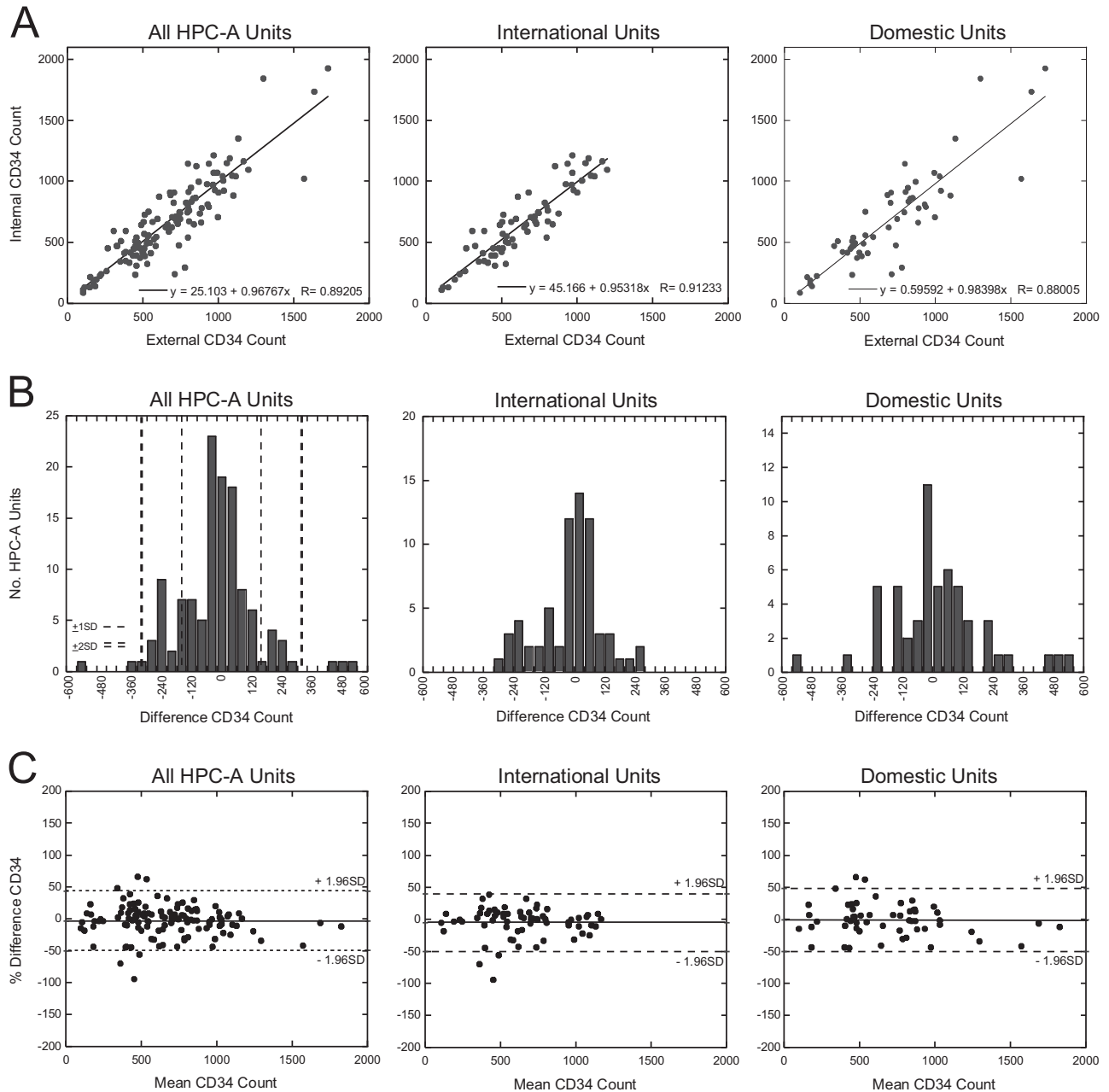


Fig. 3. Comparison of CD34 counts in allogeneic HPC-A products by donor center location. (A) Correlation between internal and external CD34 count ($\times 10^3$). (B) Absolute difference in CD34 count (external – internal). Vertical lines indicate $\pm 1SD$ (---) and $\pm 2SD$ (==). (C) 95% LA for CD34 counts. Percent (%) difference CD34 ($\pm 1.96 SD$, hatched line) plotted against the mean CD34 count from both facilities.

have confirmed the importance of sufficient CD34 cells to ensure adequate long-term engraftment.^{22,26-28} In marrow transplantation, TNC is often used as an intraoperative surrogate assessment of harvest efficacy, while both TNC and CD34 cell dose are correlated with transplant outcomes.^{26,27} Likewise, both TNC and CD34 count are important for determining the quality of umbilical cord blood for cryopreservation and transplantation.²⁸ In

donors undergoing peripheral blood stem cell collection, the number of circulating CD34 cells determine the timing of collection and the number of procedures required.²⁹ As a result, TNC, CD34 and cell viability are considered critical analytes subject to PT.^{1,2}

In general, PT for TNC and WBC counts is relatively easy given the reproducibility and precision of current automated cell analyzers.^{30,31} In contrast, CD34 PT has

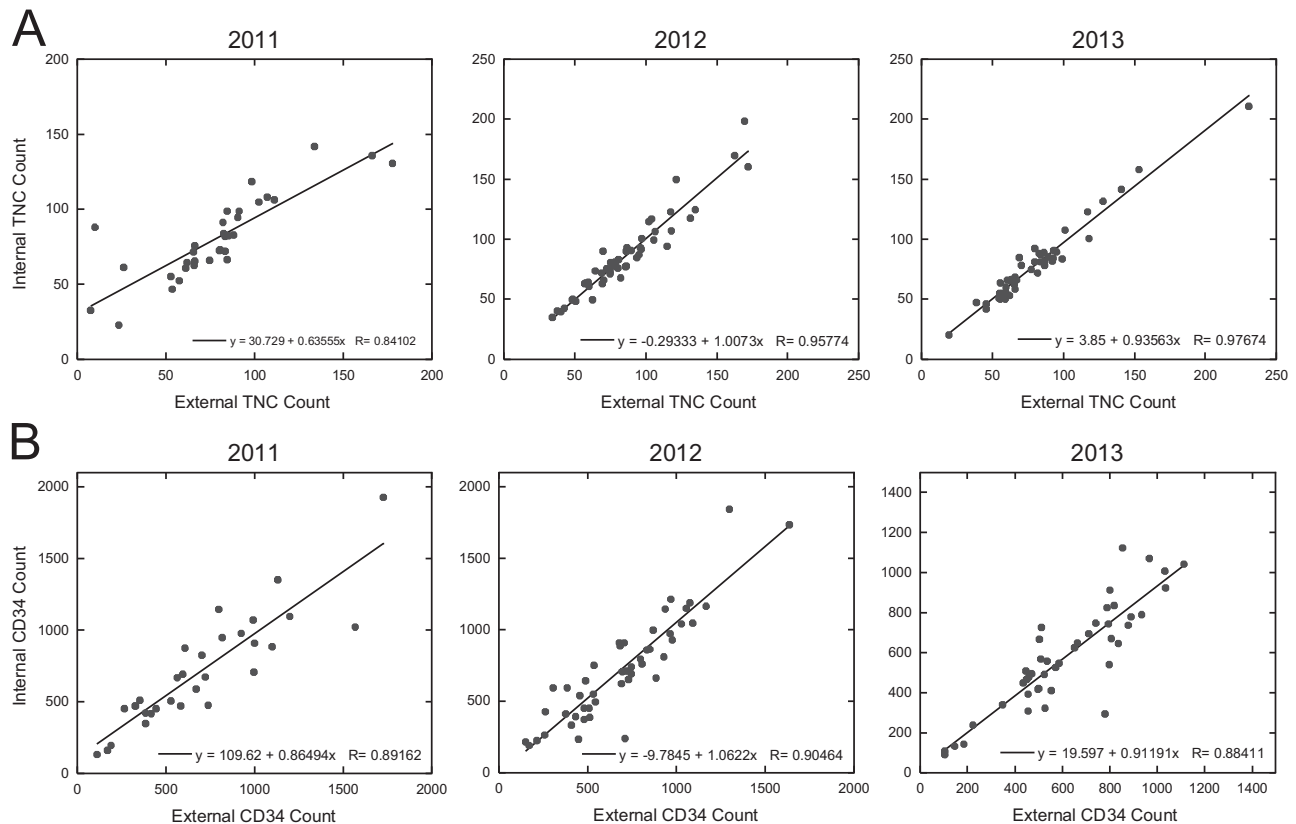


Fig. 4. Comparison of TNC (A) and CD34 (B) count by calendar year.

proved particularly challenging due to the complexity of testing and a host of preanalytical and technical factors that can separately, and synergistically, influence test results.^{12-19,32} Moreover, CD34 enumeration is a rare event analysis, which presents additional difficulties for QC, precision, and accuracy.³³ Over the past 20 years, cell therapy PT challenges have been instrumental in identifying many technical and reagent factors that can impact CD34 testing and serve as the basis for today's current best practices. These include the use of Class II and Class III anti-CD34 monoclonal antibody, preferably as CD34-PE conjugate, a multiparameter sequential gating strategy, the importance of acquiring sufficient data events, and inclusion of viability staining.^{13,14,16,17,19} Single-platform testing, which requires the addition of fluorescent beads to samples, is also reported to increase accuracy since it allows a direct internal measure of the number of CD34 cells per volume tested.^{12,13,17,33} Dual-platform testing is felt to be less accurate although studies have reported equivalent results when ISHAGE gating is used.^{14,34}

Preanalytical variables also influence CD34 PT performance and may account for 40% of the variation observed between participants.²⁰ One important preanalytical factor is the type of sample: PT samples prepared from HPC-A, which are enriched for CD34 cells, tend to have closer agreement than peripheral blood samples.^{20,34} Marrow is

also prone to high variability due to heterogeneity in CD34 staining and cell granularity.³⁴ Samples for CD34 PT have included stabilized, commercial CD34+ tissue culture line (KG1)³⁵; stabilized CD34+ acute myeloid leukemia cells¹³; heparinized whole blood²⁰; blood diluted with donor plasma³²; peripheral blood containing a cell preservative¹²⁻¹⁶; fresh HPC-A or marrow diluted in phosphate-buffered saline²⁰ or tissue-culture media (RPMI, X-VIVO)^{3-10,18}; and thawed, cryopreserved HPC-A resuspended in tissue culture media (RPMI, Dulbecco's).^{16,18}

Other preanalytical factors are the homogeneity of the samples during central processing and aliquoting, sample stability and reproducibility during storage and transport, and sample processing upon receipt.³⁶ Long delays or improper storage during transport can impact cell content between participating centers,¹⁸ particularly if cells were in the early stages of apoptosis. Studies have shown that 7-AAD, which only measures membrane integrity, is unable to detect cells in early apoptosis and/or poor proliferative capacity.^{16,37,38} To improve sample stability, some manufacturers add or collect blood in a stabilizing agent.¹²⁻¹⁷ In the United States, CAP-SCP PT samples are prepared from either peripheral blood or HPC-A, resuspended in heparinized X-VIVO 10, a serum-free hematopoietic cell medium marketed for CD34 and lymphocyte cell cultures that is supplemented with 10%

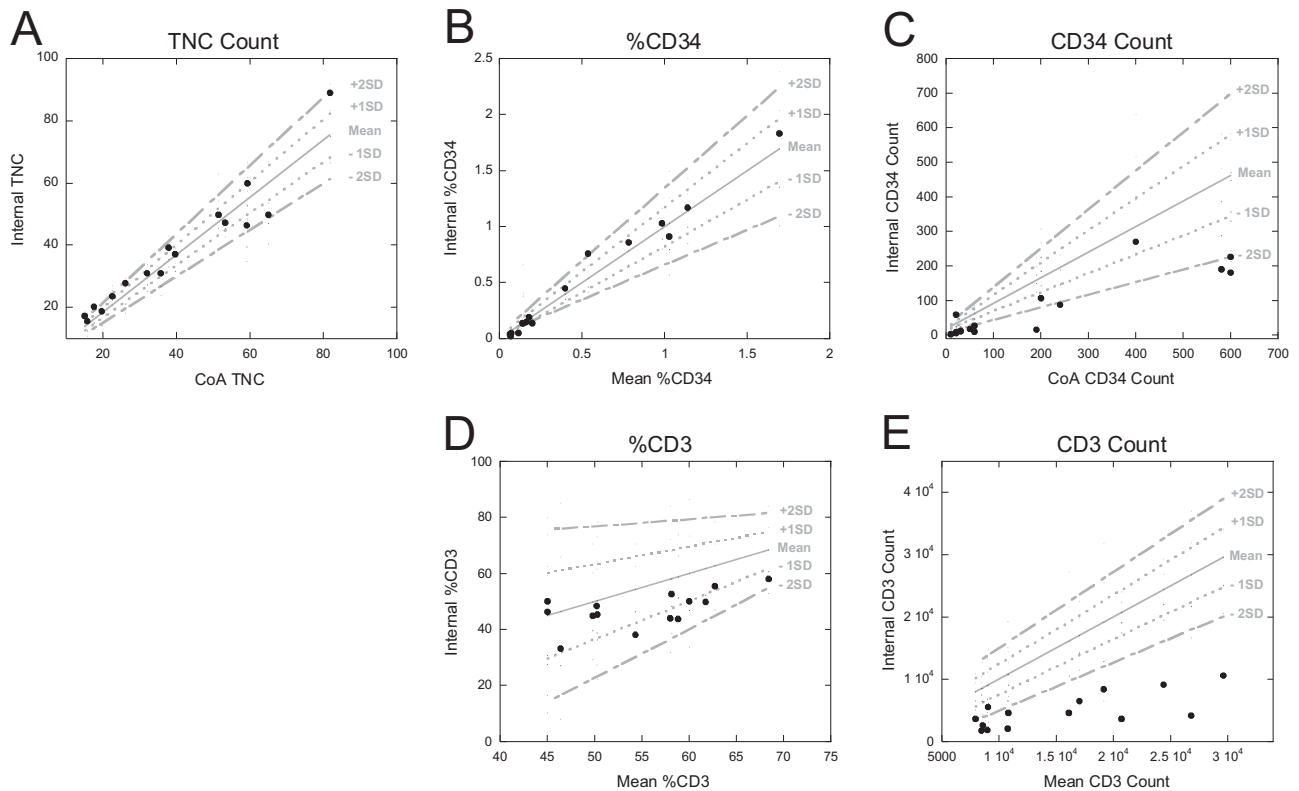


Fig. 5. Internal performance with commercial cell therapy PT challenges. (A) Internal TNC against TNC listed on CoA. (B) Internal %CD34 against mean %CD34 for dual platform. (C) Internal absolute CD34 count against CD34 count listed on CoA. (D) Internal %CD3 against mean %CD3 for dual platform. (E) Internal absolute CD3 count against the mean CD3 count. Gray lines show the mean (—, solid line), $\pm 1SD$ (···, dotted line), and $\pm 2SD$ (- - -, hatched line) for dual-platform users.

autologous serum.³⁹ Finally, laboratory differences in sample processing (cell lysis reagent, wash/no wash) can introduce additional sample variation before analysis.^{14,20,40}

Our data suggest that allogeneic HPC-A products can also serve as a PT challenge, with many advantages over commercial PT samples. HPC-A samples are large volume and significantly less subject to aliquot and sampling error. In addition, HPC-A are not subject to additives that may alter cell characteristics. Moreover, HPC-A products are packaged and transported using an established validated method to ensure cell viability and stability.

Allogeneic HPC-A are still subject to interlaboratory variability due to sample processing and technical factors including testing platform, instrumentation and software, staining reagents, pipetting, gating strategy, number of acquired events, and operator experience.^{12-18,20} Nonetheless, we observed a satisfactory performance between our results and the majority of external sites. Overall, the median percent difference for TNC (-0.15%) and CD34 (-1.1%) count was very low, with 85% TNC and 59% CD34 count falling within $\pm 10\%$ of the external facility. These results compare favorably to the UK NEQA PT program, in which participants are expected to fall within the

median 50% (25th-75th percentile) $\pm 15\%$ over three successive challenges.^{14,17}

Our experience using allogeneic HPC-A products as a paired PT challenge was significantly better than CAP-SCP PT challenges. TNC using the cell counter (XE-5000, Sysmex) was slightly lower than the CoA but still within $\pm 2SD$. Likewise, the results for %CD34 fell within $\pm 1SD$ for institutions using dual-platform testing. In contrast, we consistently had absolute CD34 values that fell near or below $2SD$, even after reanalysis without 7-AAD gating per UK NEQA guidelines.¹⁷ A comparison of %CD3 and absolute CD3 counts during the same challenges also showed significantly lower %CD3 and absolute CD3 counts. Altogether, we believe that the lower mean TNC, %CD3, and absolute CD3 suggest some sample degradation. Stability studies with the cell counter (XE-5000, Sysmex) have shown a 4% decrease in lymphocyte count within 24 hours at cool temperatures.⁴¹ Probable sample degradation was also evident during flow cytometric analysis in many samples. Sample degradation would also contribute to our inability to reach the minimum number of CD34 events in 18% of samples.¹⁹

In addition to preanalytical factors, there were three technical differences that could also contribute to the

variability we observed with commercial PT samples. One is our use of a newer multichannel (10-color) flow cytometer, which was an outlier among CAP participants. Both flow cytometry instrumentation and analytical software are variables effecting CD34 PT and lymphocyte subtyping.^{14,42} Furthermore, we use a modified ISHAGE protocol that includes CD14-FITC and a different CD45 fluorochrome (electron-coupled dye). The inclusion of CD14 for gating is recommended in the SIHON protocol developed in the Netherlands.^{14,20-22} Gating for CD34+ CD14- cells excludes nonspecific CD34 binding by Fc γ receptors on monocytic cells, which are up regulated by G-CSF and GM-CSF, as well as CD34+ CD14+ early monocytes present in marrow and peripheral blood (5%-10%).^{20-22,34} It is reported that the addition of CD14 in cord cell analysis can decrease the %CD34 cells by 0.9% to 47%.²¹ Similarly, Brecher and coworkers¹⁸ reported that institutions using CD14 had a lower %CD34 in 40% to 80% of PT samples. In contrast, Levering and coworkers¹⁴ found no significant difference in CD34 results between ISHAGE and SIHON gating strategies after reviewing the results of 64 PT samples. Likewise, our %CD34 was very close to the mean for dual-platform users in CAP-SCP challenges (Fig. 5B). Finally, we observed a very good concordance in CD34 enumeration between our center and other facilities, with a slight positive bias toward higher internal CD34 counts (median, 1%) using our modified ISHAGE protocol.

Some of the earliest CD34 PT challenges were plagued by wide interlaboratory variability, especially with fresh samples.^{18,20,32,35} Chang and Ma,³² reporting on an Australasian PT challenge using samples diluted in human plasma, showed that 65% of centers were outside the recommended range ($\pm 10\%$ median). In an early PT challenge involving 21 samples and 10 participating centers in North America, the CV for %CD34 ranged from 3.7% to 159%.¹⁸ Even when participants were provided both reagents and a standard gating protocol, CVs for %CD34 ranged from 34% to 106% due to nontechnical factors.²⁰ A survey of current CAP-SCP challenges shows similar variability: The CV for absolute CD34 count ranges from 14% to 59.4% for dual platform and 11% to 54.5% for single platform.³⁻¹⁰

Better results are reported using stabilized and/or preserved samples, often coupled with central review of LMD files and gating strategies for poor performing laboratories.^{13,14,16,17} The former New York State Department of Health CD34 PT program, which used short-term stabilized cell samples, was able to progressively improve PT challenge performance.¹⁶ Likewise, the UK NEQA program prepares and distributes preserved samples that stably retain CD34 cell expression for up to a year.^{12,13,17} As a result, the UK NEQA program has decreased variability to CV of less than 10%.^{13,17} The Netherlands has also converted to the use of long-term stabilized samples for their CD34 PT program with a significant decrease in

variability.¹⁴ The use of stabilized PT samples, however, does have some caveats.¹⁷ The stabilizer impacts cell permeability, affecting both SS and 7-AAD staining. As a consequence, participants are advised to exclude 7-AAD from gating and extend the SS gate.¹⁷ Conversely, the ability of preservation to manufacture 7-AAD+, CD34+, CD45+ cells can be exploited for QC. Gutensohn and colleagues validated the use of commercial, preserved CD34+ cells as an internal positive control for routine CD34 analysis.

In summary, we have demonstrated that allogeneic HPC-A products can serve as an external PT challenge for TNC and CD34 enumeration. HPC-A products may be a more accurate assessment of laboratory proficiency than some commercial PT samples, which are subject to preanalytic variation due to sample preparation techniques, sample homogeneity, and sample stability.³³


CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

REFERENCES

- Standards for cell therapy product services. 3rd ed. Bethesda (MD): AABB Press; 2008.
- FACT-JACIE international standards for hematopoietic cellular therapy product collection, processing and administration. 6th ed. Omaha (NE): Foundation for the Accreditation of Cellular Therapy; 2015.
- College of American Pathologists. SCP-A stem cell processing participant summary. Chicago (IL): College of American Pathologists; 2012.
- College of American Pathologists. SCP-B stem cell processing participant summary. Chicago (IL): College of American Pathologists; 2012.
- College of American Pathologists. SCP-A stem cell processing participant summary. Chicago (IL): College of American Pathologists; 2013.
- College of American Pathologists. SCP-B stem cell processing participant summary. Chicago (IL): College of American Pathologists; 2013.
- College of American Pathologists. SCP-A stem cell processing participant summary. Chicago (IL): College of American Pathologists; 2014.
- College of American Pathologists. SCP-B stem cell processing participant summary. Chicago (IL): College of American Pathologists; 2014.
- College of American Pathologists. SCP-A stem cell processing participant summary. Chicago (IL): College of American Pathologists; 2015.
- College of American Pathologists. SCP-B stem cell processing participant summary. Chicago (IL): College of American Pathologists; 2015.

11. Bellisimo DB; American College of Medical Genetics. Practice guidelines and proficiency testing for molecular assays. *Transfusion* 2007;47(1 Suppl):79S-84S.
12. Barnett D, Granger V, Storie I, et al. Quality assessment of CD34+ stem cell enumeration: experience of the United Kingdom National External Quality Assessment Scheme (UK NEQAS) using a unique stable whole blood preparation. *Br J Haematol* 1998;102:553-65.
13. Barnett D, Granger V, Kraan J, et al. Reduction of intra- and inter-laboratory 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.
14. Levering WH, Preijers FW, van Wieringen WN, et al. Flow cytometric CD34+ stem cell enumeration: lessons from nine years' external quality assessment with the Benelux countries. *Cytometry B Clin Cytom* 2007;72:178-88.
15. Lysak D, Kalina T, Martinek J, et al. Interlaboratory variability of CD34+ stem cell enumeration. A pilot study to national external quality assessment within the Czech Republic. *Int J Lab Hematol* 2010;32:e229-36.
16. López MC, Lawrence DA. Proficiency testing experience for viable CD34+ stem cell analysis. *Transfusion* 2008;48:1115-21.
17. Whitby A, Whitby L, Fletcher M, et al. ISHAGE protocol: are we doing it correctly? *Cytometry B Clin Cytom* 2012;82:9-17.
18. Brecher ME, Sims L, Schmitz J, et al. North American multicenter study on flow cytometric enumeration of CD34+ hematopoietic stem cells. *J Hematother* 1996;5:227-36.
19. Sutherland DR, Anderson L, Keeney M, et al. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. *J Hematother* 1996;5:213-26.
20. Gratama JW, Kraan J, Levering W, et al. Analysis of variation in results of CD34+ hematopoietic progenitor cell enumeration in a multicenter study. *Cytometry* 1997;30:109-17.
21. Ho HT, Lin CC, Lin J. Variation of CD34+ cell enumeration of cord blood with additional CD14 gating by flow cytometry. *Lab Med* 2003;34:481-5.
22. Weaver CH, Hazelton B, Birch R, et al. An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. *Blood* 1995;86:3961-9.
23. Kim SY, Kim JE, Kim HK, et al. Accuracy of platelet counting by automated hematologic analyzers in acute leukemia and disseminated intravascular coagulation. *Am J Clin Pathol* 2010;134:634-47.
24. Bland JM, Altman DG. Applying the right statistics: analyses of measurement studies. *Ultrasound Obstet Gynecol* 2003;22:85-93.
25. Bisset LR, Lung TL, Kaelin M, et al. Reference values for peripheral blood lymphocyte phenotypes applicable to the healthy adult population in Switzerland. *Eur J Haematol* 2004;203-12.
26. Barrett AJ, Ringdén O, Zhang MJ, et al. Effect of nucleated marrow cell dose on relapse and survival in identical twin bone marrow transplants for leukemia. *Blood* 2000;95:3323-7.
27. Morariu-Zamfir R, Rocha V, Devergie A, et al. Influence of CD34(+) marrow cell dose on outcome of HLA-identical sibling allogeneic bone marrow transplants in patients with chronic myeloid leukaemia. *Bone Marrow Transplant* 2001;27:575-80.
28. Cohen YC, Scaradavou A, Stevens CE, et al. Factors affecting mortality following myeloablative cord blood transplantation in adults: a pooled analysis of three international registries. *Bone Marrow Transplant* 2011;46:70-6.
29. Gambell P, Herbert K, Dickinson M, et al. Peripheral blood CD34+ cell enumeration as a predictor of apheresis yield: an analysis of more than 1,000 collections. *Biol Blood Marrow Transplant* 2012;18:763-72.
30. Meintker L, Ringwald J, Rauh M, et al. Comparison of automated differential blood cell counts from Abbot Sapphire, Siemens Advia 120, Beckman Coulter DxH 800, and Sysmex XE-2100 in normal and pathologic samples. *Am J Clin Pathol* 2013;139:641-50.
31. Van den Bossche J, Devreese K, Malfait R, et al. Reference intervals for a complete blood count determined on different automated hematology analysers: AbxPentra 120 Retic, Coulter Gen-S, Sysmex SE 9500, Abbot Cell Dyn 4000 and Bayer Advia 120. *Clin Chem Lab Med* 2002;40:69-73.
32. Chang A, Ma DD. The influence of flow cytometric gating strategy on the standardization of CD34+ cell quantitation: an Australian multicenter study. Australian BMT Scientists Study Group. *J Hematother* 1996;5:605-16.
33. Preti RA, Chan WS, Kurtzberg J, et al. Multi-site evaluation of the BD stem cell enumeration kit for CD34+ cell enumeration on the BD FACSCanto II and BD FACSCalibur flow cytometers. *Cytotherapy* 2014;16:1558-74.
34. Gajkowska A, Oldak T, Jastrzevska M, et al. Flow cytometric enumeration of CD34+ hematopoietic stem and progenitor cells in leukapheresis product and bone marrow for clinical transplantation: a comparison of three methods. *Folia Histochem Cytobiol* 2006;44:53-60.
35. Serke S, Huhn D. Circulating CD34-expressing cells: German proficiency testing survey. *J Hematother* 1998;7:37-43.
36. De la Salle BJ, McTaggart PN, Briggs C, et al. The accuracy of platelet counting in thrombocytopenic blood samples distributed by the UK National External Quality Assessment Scheme for General Hematology. *Am J Clin Pathol* 2012;137:65-74.
37. Patterson J, Moore CH, Palser E, et al. Detecting primitive hematopoietic stem cells in total nucleated and mononuclear cell fractions from umbilical cord blood segments and units. *J Transl Med* 2015;13:94.
38. van Haute I, Lootens N, De Smet S, et al. Viable CD34+ stem cell content of a cord blood graft: which measurement performed before transplantation is most representative? *Transfusion* 2004;44:547-54.

39. Lonza [Internet]. Homepage [cited 2016 Oct 1]. Available from: www.Lonza.com.
40. Einwallner E, Subasic A, Strasser A, et al. Lysis matters: red cell lysis with FACS Lyse affects the flow cytometric enumeration of circulating leukemic blasts. *J Immunol Methods* 2013;390:127-32.
41. Joshi A, Mcvicker W, Segalla R, et al. Determining the stability of complete blood count parameters in stored blood samples using the Sysmex XE-5000 automated haematology analyser. *Int J Lab Hematol* 2015;37:705-14.
42. Asman M, Sachdeva N, Davila L, et al. Influence of 4- and 6-color flow cytometers and acquisition/analysis softwares on the determination of lymphocyte subsets in HIV infection. *Cytometry B Clin Cytom* 2007;72:380-6.
43. Gutensohn K, Jessen M, Ketels A, et al. Flow cytometric analyses of CD34+ cells with inclusion of internal positive controls. *Transfusion* 2012;52:284-90. 

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. HPC-A units with discrepant counts (% difference 50% or greater).