Use of Allogeneic Apheresis Stem Cell Products as an Inter-Laboratory Proficiency Challenge

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ABSTRACT

Introduction: 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 inter-laboratory exchange. We investigated the utility of allogeneic hematopoietic progenitor cell apheresis products (HPC-A) as an inter-laboratory PT challenge for total nucleated cell (TNC) and CD34 assessment.

Methods: Three-year retrospective and comparative review of unrelated allogeneic HPC-A products received by the University of Michigan between 1/2011 and 12/2013. Internal TNC and CD34 counts were compared to the external collecting facility by paired t-test and linear regression. The absolute and % 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 inter-laboratory PT exchange for all critical analytes, including TNC and CD34 counts, 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.

INTRODUCTION

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.[2] AABB Standards for Cell Therapy Services are more specific, mandating participation in a proficiency test (PT) program for each measured analyte (8.2).[1] Moreover, US-based laboratories are required to participate in a CMS-approved PT program for each CLIA-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 non-graded 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 media supplemented with 10% human sera.[3-10] Participants are asked to measure the TNC count, 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 inter-laboratory 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 NMDP can be considered an inter-laboratory 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 re-suspension in tissue culture media.[3-10,12-18] To assess the utility of allogeneic products for PT, we compared the TNC and CD34 counts from 141 peripheral blood hematopoietic progenitor cell apheresis units (HPC-A) received at our institution from external collection facilities over a 3-year period.

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 counts were compared and analyzed as an inter-laboratory PT challenge. Data from the external donor facility included the absolute TNC ($x10^9$) and CD34 count ($x10^6$) of each unit, product volume (mL), whether it was collected by a National Marrow Donor Program (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 counts, cell viability, % MNC, hematocrit (%), platelet count ($10^3/\mu L$) and transplant cell dose.

Internal Cell Analysis

Allogeneic products were tested for TNC count, 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, Japan). The WBC differential was determined by manual methods. Per protocol, samples with an initial WBC count over 300,000/μL were diluted 1:5 in commercial cell diluent (Cell Pack DCL, Sysmex) and re-analyzed.

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 cell lysis and immediate analysis: Samples were not subjected to a wash step or fixation prior to flow cytometry. CD34 analysis was performed using a dual-stage, 4-color modified ISHAGE (International Society for Hematotherapy and Graft Engineering) protocol on a Gallios multichannel flow cytometer (Beckman Coulter, Indianapolis, IN). Per ISHAGE, CD34 cells were identified through sequential gating using CD45, CD34, forward and side scatter (SS) to identify CD34+, $CD45^{dim}$, SS^{low} HPC cells.[17,19] In addition, samples were co-stained with an anti-CD14 to exclude CD34+, CD14+ cells.[14, 20-22] Cell viability was determined with 7-AAD (Life Technologies, Carlsbad, CA). 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 (ECD) 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 for flow cytometry included commercial reagents for CD34 and CD45 (Chex CD-Plus BC and CD-Chex CD34; Streck, Omaha, NE).

Statistics:

The TNC and CD34 counts 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 ± standard deviation (x ± SD). The 95% limit of agreeability (95% LA) were calculated and plotted as described.[23,24] Specifically, the % difference was plotted against the mean absolute CD34 count ([external count + internal count] ÷ 2). Results falling between the x ± 1.96SD 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 Kaleidograph (Synergy Software, Reading, PA). Catagorical variables were compared by chi square using EpiInfo (Centers for Disease Control, Atlanta, GA).

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 sterilely 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 day of receipt per institutional protocols as described above. All samples were tested within 24 hours of receipt per CAP requirements.

TNC and WBC count were performed on Sysmex XE-5000. CD34 analysis was performed using a modified ISHAGE protocol as described above and reported as %CD34 and CD34 count (x10^6). In addition, the list mode data (LMD) files were re-analyzed 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 manufacturer's Certificate of Analysis (CoA) as provided by CAP.[3-10] To compare our results with other participants, the mean ±1SD and ±2SD 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 (x10^6) 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 % 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 8 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 few differences relative to donor characteristics, volume, total cell counts and cell dose (Table 2).

Comparison of TNC Counts

External and internal TNC counts 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 counts, especially for domestic units.

The absolute and percent difference in TNC counts were also determined and compared (Fig. 2B, 2C). Overall, the TNC counts in 84% (108/128) units were within ±10% of each other (Fig 2C). For domestic units, there was a slight bias toward higher internal counts (64% units), although the median % difference was modest (-3.3%, Table 3). There was a trend (p=0.07) toward higher internal TNC counts 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 % difference was 1.35% and 5.1%, respectively. We also examined TNC counts 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 counts 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 counts, 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 % 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 ±10% of the collecting facility's yield in 72 units (59%), and within 20% in 94 units (77%).

When examined by donor center location, 41/55 (74.5%) domestic units were within ±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 % difference in CD34 counts for international units was -0.7% (Table 3; 95% LA: -45.5, 44.1), with nearly half falling within ±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 count 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 a10% 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 % difference in either TNC or CD34 count was > 50% (Supplemental data, 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 3 (6%) in 2012 and only 1 (2%) in 2013. All 6 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 counts, 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 (±1SD and ±2SD) for peer participants.

In general, our results for TNC counts fell within ±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%, +15.3%) but 4.9% higher than the participant mean (P=0.07; range, -9%, 6.8%). The average coefficient of variation (CV) across all TNC challenges was 9.2% ± 2.3%.

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 ($x10^6$) 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 (>75,000 CD45+ cells, >100 CD45+,CD34+ cells).[19] Although sufficient CD45+ events were collected, the minimum number of CD34+ events could not reached in 3 samples (range, 41 – 67 CD34 cells).

Because of our consistently low absolute CD34 counts, we also examined our performance with %CD3 and absolute CD3 ($x10^6$) counts during the same challenges. Unlike CD34+ cells, CD3+ cells are plentiful and account for 55-84% of all peripheral blood lymphocytes.[25] As shown in Fig 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 prior to receipt and testing.

DISCUSSION

The TNC and viable CD34 counts are critical to clinical decision-making during stem cell collection and transplantation. Numerous clinical studies over two decades have confirmed the importance of sufficient CD34 cells to ensure adequate long-term engraftment.[22,26-28] In bone marrow transplantation, TNC count is often used as an intra-operative surrogate assessment of harvest efficacy, while both TNC and CD34 cell dose are correlated with transplant outcomes.[26,27] Likewise, both TNC and CD34 counts 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 proved particularly challenging due to the complexity of testing, and host of pre-analytical 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 quality control, precision and accuracy.[33] Over the last 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 mAb, 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]

Pre-analytical variables also influence CD34 PT performance and may account for 40% of the variation observed between participants.[20] One important pre-analytical 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] Bone 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 cells;[13] heparinized whole blood;[20] blood diluted with donor plasma;[32] peripheral blood containing a cell preservative;[12-16] fresh HPC-A or marrow diluted in phosphate-buffered saline[20] or tissue-culture media (RPMI, X-VIVO^{TM});[3-10,18] and thawed, cryopreserved HPC-A resuspended in tissue culture media (RPMI, Dulbecco's).[16,18]

Other pre-analytical 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, re-suspended in heparinized X-VIVO 10, a serum-free hematopoietic cell media marketed for CD34 and lymphocyte cell cultures that is supplemented with 10% autologous serum.[39] Finally, laboratory

differences in sample processing (cell lysis reagent, wash/no wash) can introduce additional sample variation prior to 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 inter-laboratory 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 % difference for TNC (-0.15%) and CD34 (-1.1%) counts was very low, with 85% TNC and 59% CD34 counts falling within ±10% of the external facility. These results compare favorably to UK NEQA PT program, in which participants are expected to fall within the median 50% (25^{th}-75^{th} percentile) ± 15% over 3 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 counts using the Sysmex XE-5000 were slightly lower than the CoA but still within ± 2SD. Likewise, the results for %CD34 fell within ±1SD for institutions using dual platform testing. In contrast, we consistently had absolute CD34 values that fell near or below 2SD, even after re-analysis 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 counts, %CD3 and absolute CD3 suggest some sample degradation. Stability studies with the Sysmex XE-5000 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 pre-analytical 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 testing and lymphocyte subtyping.[14,42] Furthermore, we use a modified ISHAGE protocol that includes CD14-FITC and a different CD45 fluorochrome (ECD). 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 Fcγ receptors on monocytic cells, which are upregulated 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 et al reported that institutions using CD14 had a lower %CD34 in 40% to 80% of PT samples.[18] In contrast, Levering et al found no significant difference in CD34 results between ISHAGE and SIHON gating strategies after reviewing the results of 64 PT samples.[14] 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 inter-laboratory variability, especially with fresh samples.[18,20,32,35] Chang and Ma, reporting on an Australasian PT challenge using samples diluted in human plasma, showed that 65% of centers were outside the recommended range (±10% median).[32] 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 14-59.4% for dual platform and 11% - 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<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 quality control. Gutensohn et al validated the use of commercial, preserved CD34+ cells as an internal positive control for routine CD34 analysis.[43]

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]

ABBREVIATIONS:

7-AAD, 7-aminoactinomycin D; CAP-SCP, College of American Pathology – Stem Cell Processing; CoA, Certificate of Analysis; FITC, fluorescein isothiocyanate; ISHAGE, International Society for Hematotherapy and Graft Engineering; 95% LA, 95% limits of agreeability; LMD, list mode data; NMDP, National Marrow Donor Program; PE, phycoerythrin; PT, proficiency test/testing; TNC, total nucleated cell count; SS, side scatter; UK NEQA, United Kingdom National External Quality Assurance program.

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Table 1. External donor center demographics

Collection Centers	No. Centers	No. Units
All Donor Centers	40	141
International	17	69
NMDP	7	58
Non-NMDP	10	11
No. Countries	9	
Europe	14	66
Mideast	2	2
Australia	1	1
Domestic	23	72
NMDP	23	72
Non-NMDP	0	0
No. States	15	
Eastern*	8	39
Central*	4	14
Mountain*	2	12
Pacific*	1	7

* Time zone of external donor center.

Table 2. Comparison of allogeneic HPC-A Units by donor center location*

Variable	International	Domestic	P
No. Donors	65	66	-
Gender (M/F)	55/10	49/17	0.15
Age, years	33.1 ± 9.3	34.5 ± 10.3	0.40
Total Units	69	72	-
No. Split Units	4	6	-
Unit Volume, mL	360 ± 108	348 ± 109	0.53
TNC, x 10^{9*}	79.35 ± 27.56	83.78 ± 38.92	0.44
Cell Viability (%)*	93.7 ± 11.8	96.4 ± 12.0	0.17
% MNC*	68.0 ± 20.0	67.6 ± 20.2	0.90
Total MNC, x 10^{9*}	50.15 ± 14.18	52.37 ± 19.49	0.44
MNC/kg, x 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, x 10^{6*}	638.65 ± 272.42	696.71 ± 357.75	0.31
CD34/kg, x 10^{6*}	8.41 ± 5.58	9.01 ± 8.30	0.61
Hematocrit (%)*	3.9 ± 1.8	4.5 ± 2.4	0.14
Platelet, x $10^{3}/\mu L$*	2154 ± 959	2525 ± 1018	0.03

* Based on internal cell counts.

Table 3. Paired comparison of internal and external TNC and CD34 Counts*

	No. Units	Total Cell Counts		P§	External – Internal	
		External†	Internal‡		[difference]‖ (median)	% difference¶ (median)
TNC ($x10^9$)						
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 Units	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 Units	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 ($x10^6$)						
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 Units	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 Units	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 ± 267.2	564.78 ± 221.5	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 Figure 1)

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

‡ Internal absolute counts, reported as x ± 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)

FIGURE LEGENDS

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

Figure 2. Comparison of TNC counts in allogeneic HPC-A products by donor center location. A) Correlation between internal and external TNC count ($x10^8$). B) Absolute difference in TNC count (external –internal). C) Percent (%) difference in TNC count. Vertical lines indicate ±1SD (- -) and ±2SD (= =).

Figure 3. Comparison of CD34 counts in allogeneic HPC-A products by donor center location. A) Correlation between internal and external CD34 count ($x10^6$). B) Absolute difference in CD34 count (external – internal). Vertical lines indicate ±1SD (- -) and ±2SD (= =). C) 95% LA for CD34 counts. Percent (%) difference CD34 (± 1.96 SD, hatched line) plotted against the mean CD34 count from both facilities.

Figure 4. Comparison of TNC (A) and CD34 (B) counts by calendar year.

Figure 5. Internal performance with commercial cell therapy PT challenges. A) Internal TNC count against TNC count 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), ±1SD (·····, dotted line) and ±2SD (— - —, hatched line) for dual platform users.

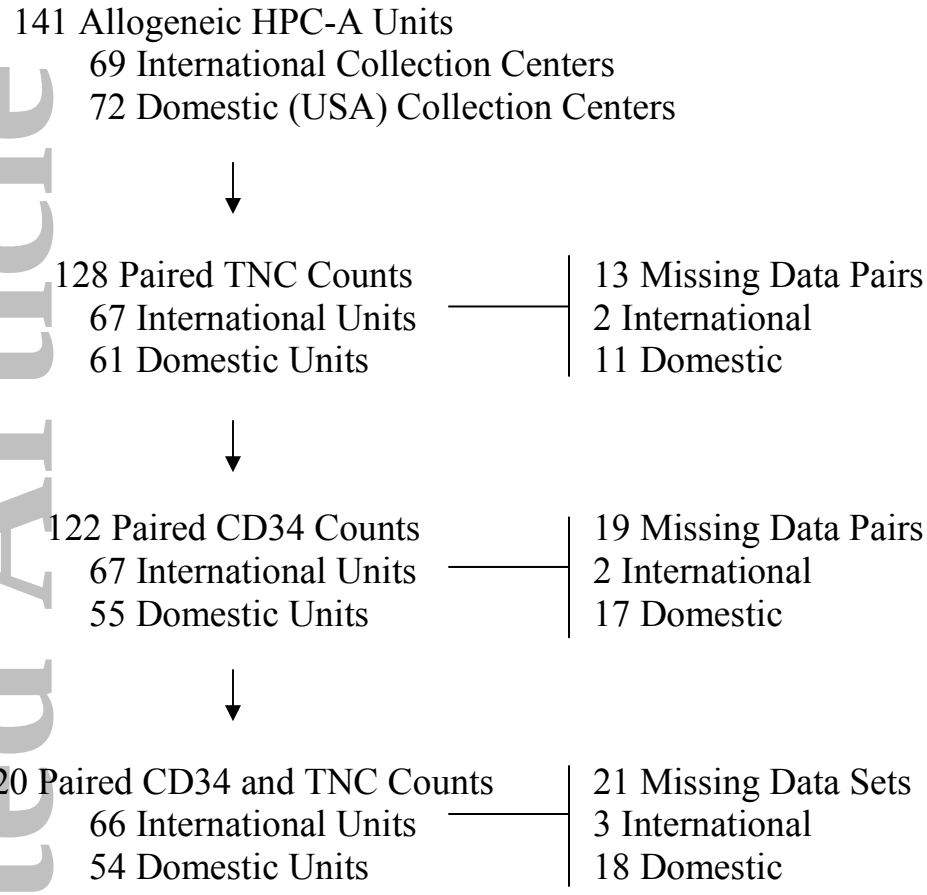

Figure 1

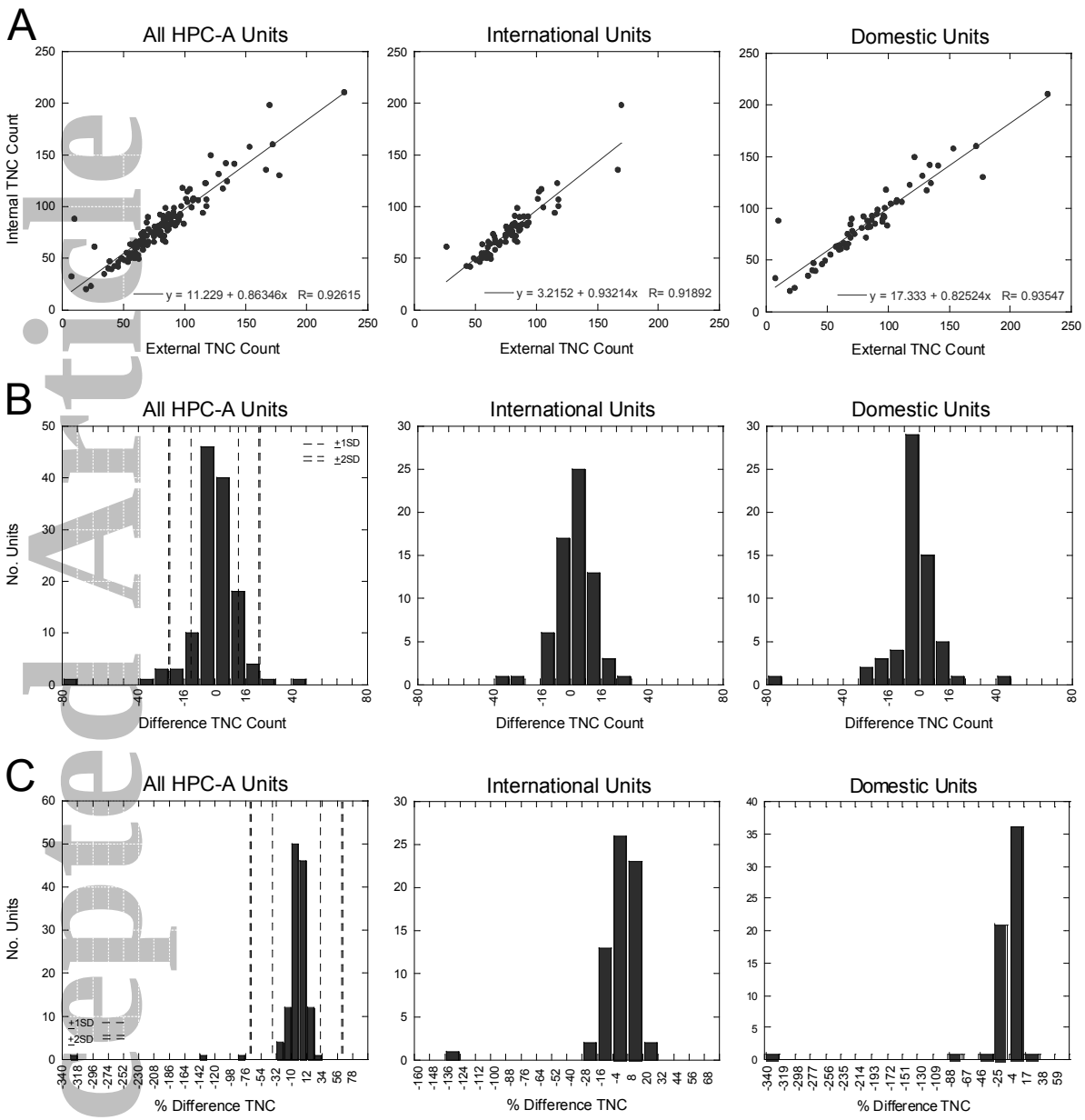

Figure 2

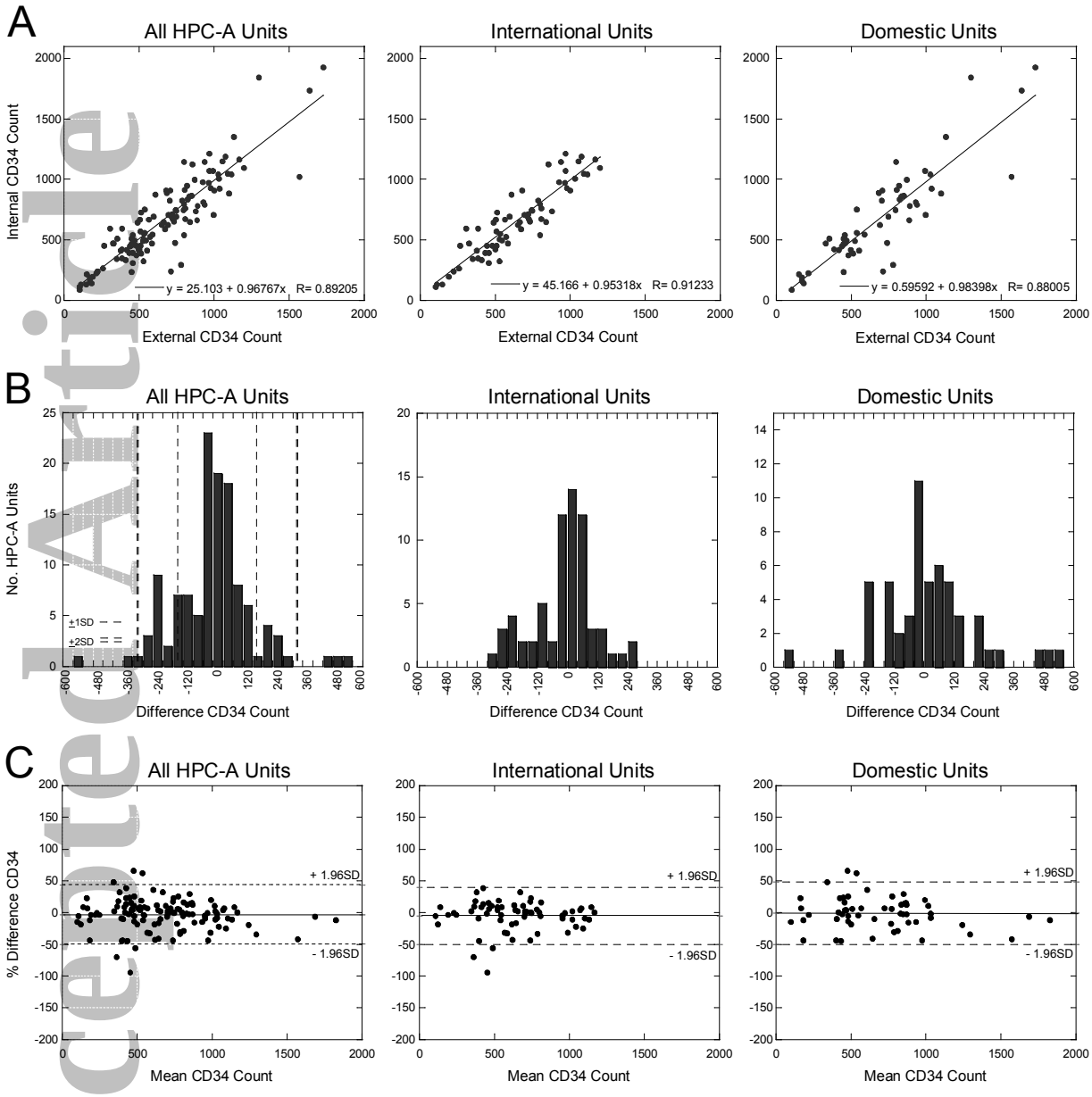

Figure 3

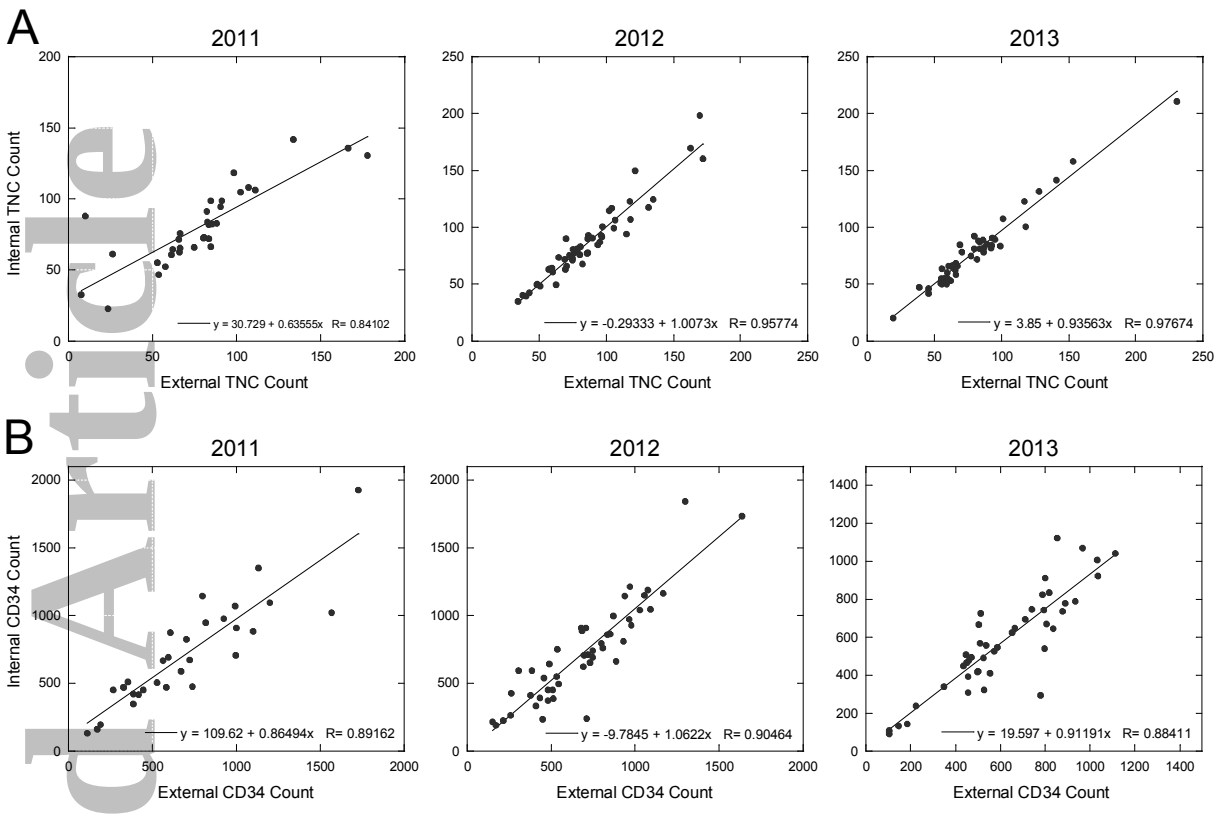

Figure 4

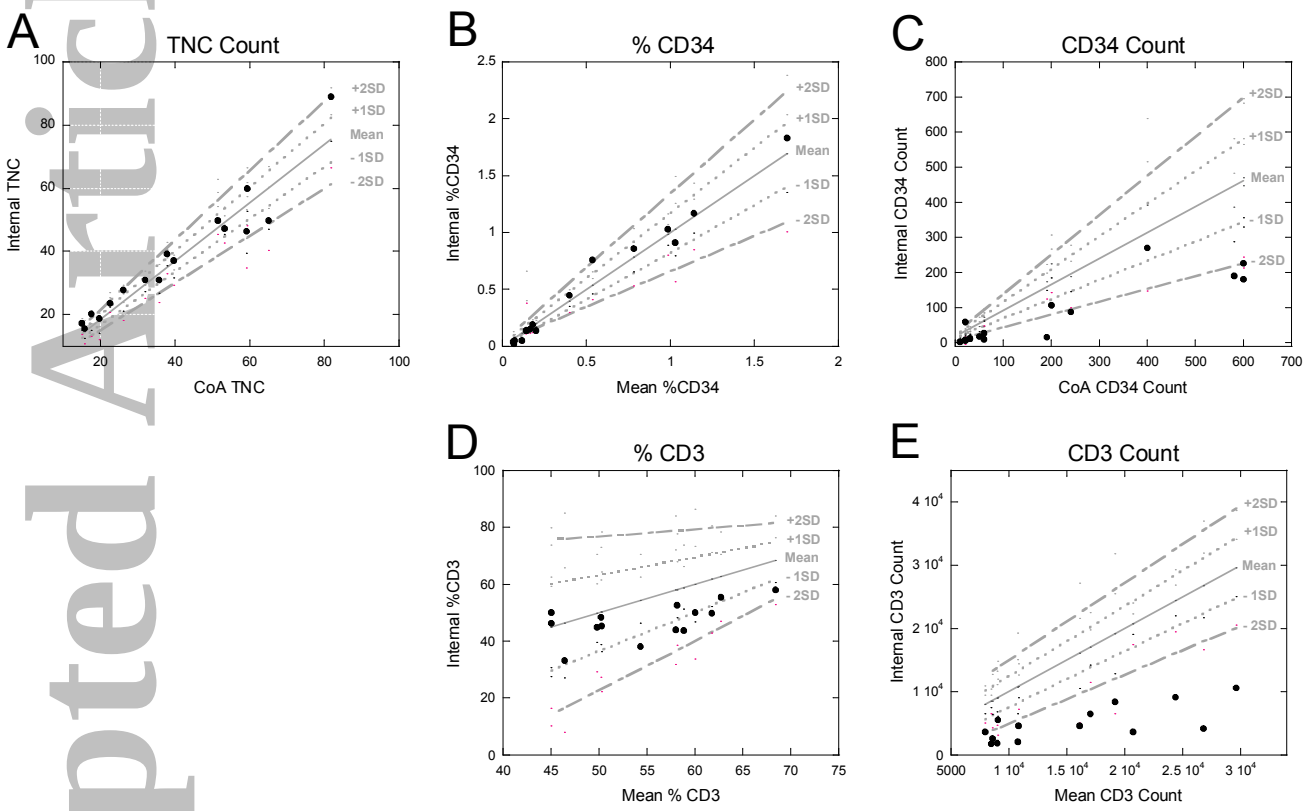

Figure 5

Supplemental Data, Table 1. HPC-A units with discrepant counts (% difference 50% or greater)*

Cell Viability	TNC Count (x 10^9)			CD34 Count (x 10^6)			Donor Center	Year
	External	Internal	% Difference*	External	Internal	% Difference*		
99%	9.84	88.02	**-79 %**	1566	1021	+35 %	Europe	2011
94%	22.99	61.33	**-136 %**	564	668.2	-18.5%	USA	2011
98%	7.60	32.56	**-382 %**	172	159.5	+ 7%	USA	2011
94%	64.51	73.39	-15 %	382.6	595.5	**-56 %**	Europe	2012
98%	162.72	169.54	-4 %	260.3	423.9	**-63 %**	Europe	2011
94%	66.13	66.50	+0.9 %	266	452	**-70 %**	Europe	2011
88%	169.73	198.50	-17%	305.5	595.5	**-94%**	Europe	2011
99%	40.18	39.65	+1.3%	447.8	223.9	**+50 %**	USA	2012
97%	95.1	89.36	-7%	778.6	294.9	**+62%**	USA	2013
99%	86.51	92.91	-7 %	709.4	241.6	**+66 %**	USA	2012

* % Difference= (External Count – Internal Count)/External Count x 100