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Manufacturing

Meeting the COVID challenge: Optimizing vCD34$^+$ in cryopreserved HPC samples for implementation of an external QA Program

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ABSTRACT

Background: The COVID-19 pandemic has forced a fundamental change in the global procurement of allogeneic hematopoietic progenitor cells (HPCs) for transplantation. To better meet the emergent challenges of transporting cryopreserved allogeneic HPC during pandemics, there is an urgent need for External Quality Assurance (EQA) programs to evaluate reproducibility and harmonization of viable CD34$^+$ cell (vCD34$^+$) HPC enumeration, as the current EQA programs are unsuitable for analysis of vCD34$^+$. The cost-effective distribution of HPC cryopreserved reference samples (CRSs) with acceptable reproducibility and specificity is key to the success of a vCD34$^+$ EQA program.
Methods: Cryopreserved HPC samples (n = 11) were either stored on dry ice for 1 to 4 days or for 1 day followed by liquid nitrogen (LN) storage for 1 to 3 days to assess optimal conditions for vCD34$^+$ EQA. Flow cytometric enumeration of vCD34$^+$ HPCs was performed using a single platform assay combined with 7-AAD viability dye exclusion. The optimum transportation condition was validated in pilot and multicenter national studies (n = 12).
Results: A combination of 1 day on dry ice followed by LN storage stabilized viability compared with continuous storage on dry ice. This study demonstrates that dispatch of CRSs on dry ice to recipient centers across a distance of $\leq$4000 km within 26 h, followed by LN storage, resulted in reproducible intercenter vCD34$^+$ enumeration. The estimated cost of safer and more convenient dry ice delivery is $>$20-fold lower than that of LN.
Conclusion: This approach can form the basis for economically and scientifically acceptable distribution of CRSs for external vCD34$^+$ EQA.

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Introduction

The COVID-19 pandemic has fundamentally changed the global procurement of voluntary unrelated and related allogeneic hematopoietic progenitor cells (HPCs) for transplantation. Pre-pandemic, allogeneic donor HPCs were transported as fresh material using personal couriers on passenger flights. The global passenger aviation slowdown necessitated a rapid change in practice to the cryopreservation of allogeneic HPCs at the collection facility, with subsequent dry shipper transport on freight aircraft to the transplant center. Reference vials of the cryopreserved HPC product that accompany the package serve as a source of quality control material for assessment of engraftment potential at the transplant facility. A recent report [1] found highly variable vCD34$^+$ HPC recoveries in cryopreserved allogeneic HPCs procured by national and international centers, with implications for patient safety after the rapid switch from courier-transported fresh HPCs to cryopreserved product because of COVID-19 travel restrictions. This finding illustrates the urgent need to establish External Quality Assurance (EQA) programs for vCD34$^+$ HPC enumeration, and a major barrier is the expense and safety of gold-standard transport of CRSs using a liquid nitrogen (LN) shipper.

Rapid enumeration of CD34$^+$ HPCs by flow cytometry is the accepted biomarker of engraftment potential [2] and also facilitates monitoring and optimal harvest timing of allogeneic and autologous blood HPCs by apheresis. Since the establishment of a widely accepted exclusion gating protocol [3], considerable standardization of methodology has occurred, enabling interlaboratory comparisons [4]. Inclusion of a viability dye such as 7-aminoactinomycin D (7-AAD) allows enumeration of viable CD34$^+$ cells (vCD34$^+$), which is particularly useful for cryopreserved samples [5].

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EQA programs use a common reference sample to identify underperforming laboratories and enable targeted interventions and training to gain conformant outcomes in previously nonconforming facilities. National and international EQAs use fresh or stabilized cells for enumeration of total $CD34^+$ to ensure that results in fresh HPC harvests are accurate, reproducible between centers and valid for clinical application [6–8]. Global participation in these external EQAs was enabled by the relatively low distribution costs of stable reference material at ambient temperature across a geographic expanse. However, the use of stabilized cells in an EQA precludes enumeration of $vCD34^+$ HPCs.

The FACT-JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing and Administration (Eighth Edition) [9] requires that, " for HPC products intended for restoration of hematopoiesis, an assay measuring viable CD34 shall be performed." Furthermore, there is a requirement for facilities to have a program, such as $vCD34^+$ HPC cell assessment, that regularly evaluates the viability and potency of cryopreserved cellular therapy products.

There are no acceptable commercial quality control materials or interlaboratory standardization programs for $vCD34^+$ HPC enumeration on fresh or cryopreserved samples. Fresh samples must be stabilized before distribution, which makes these samples unsuitable for viability assessment using 7-AAD. Cryopreserved reference samples (CRSs) are generally not used due to the logistics and cost implications of transport to participating facilities. Although comparison studies between laboratories within close proximity may use a bulky LN dry shipper to transport CRSs, this method is expensive and impractical for interstate or international participants. An alternative low-temperature method is dry ice transport to allow the establishment of lower-cost external EQAs to better meet the needs of autologous HPC programs and the emergent challenges of transporting cryopreserved allogeneic HPCs during the COVID-19 pandemic.

This study evaluated the feasibility of distribution of CRSs on dry ice for $vCD34^+$ enumeration. After initial simulation and pilot studies, a multicenter study was conducted in 12 laboratories across Australia, covering distances of $\leq$4000 km ($\sim$2500 miles) and transit times of $\leq$26 h. Shipping on dry ice, with return to storage in vapor-phase LN on receipt, facilitated nationwide delivery to testing laboratories of analytically comparable CRSs, with a >20-fold reduction in cost compared with transportation in an LN dry shipper. This approach may facilitate the establishment of an urgently needed, practical and cost-effective EQA program for $vCD34^+$ HPC enumeration.

Methods

HPC sample preparation

Approval was obtained from the St Vincent's Hospital Human Research Ethics Committee (10/070) to collect G-CSF–mobilized HPCs from autologous and allogeneic donors after receiving informed consent according to the Declaration of Helsinki. When the harvested yield of HPCs was greater than that required for transplantation, a 15-mL sample was obtained from each of the 10 HPC harvests (denoted A to J), diluted in autologous plasma and 10% DMSO at a cell count $<250 \times 10^9$/L, aliquoted (300 to 500 μL per 1.8-mL cryovial) and immediately cryopreserved in a Planer Kryo 360-3.3 controlled-rate freezer at -1°C/min to -50°C, then -5°C/min to -180°C. Vials were transferred to the vapor phase of LN for storage.

Evaluation of storage conditions on $vCD34^+$

To simulate dry ice and LN transport and storage conditions, cryopreserved vials of harvested HPCs were stored under different conditions (Table 1) at the central laboratory, after which $vCD34^+$ enumeration was performed.

Enumeration of $vCD34^+$ HPCs

Vials were thawed in a 37°C water bath with gentle mixing and 50 μl was immediately diluted 1/10 in DAS (5% Dextran 40, 2.5% Human Serum Albumen, 0.9% saline). 100 μl of diluted sample ($\sim 1 - 2.5 \times 10^6$ cells), was transferred to duplicate Trucount tubes (Becton Dickinson) containing 20 μl CD45-FITC/CD34-PE (Becton Dickinson) and 20 μl 7-AAD viability dye (Becton Dickinson). Tubes were gently mixed and incubated in the dark at room temperature for 10 minutes. Each tube was diluted with 500 μl DAS and the sample acquired with a FACSCanto II (8 or 10 color) flow cytometer (Becton Dickinson). Data files were analyzed using FACSDiva software (Becton Dickinson). The single platform exclusion gating strategy developed by Sutherland and Keeney [3, 5] was used to enumerate the $vCD34^+$ HPC population.

To determine the effect of different storage conditions on vCD34 HPC, the reference point used was the mean $vCD34/\mu$l of the liquid nitrogen control (LNC) tested on Day-1 to Day-4 of each HPC harvest. The percent change in vCD34 of each storage condition from the LNC for the same HPC was calculated using the formula

$$(vCD34^+ per\ \mu L \text{ of storage condition} - vCD34^+ \text{per } \mu L \text{ of the LNC mean}) / vCD34^+ \text{per } \mu L \text{ of the LNC mean} \times 100.$$

Pilot study

To extend the findings of the evaluation study, a pilot study was initiated with 2 HPC harvests (HPC-E and HPC-G). Two vials from each harvest were packed in $\sim$1.5 kg dry ice (day 0) and transported by overnight air freight from the central laboratory (Lab A) to 2 interstate laboratories (Labs B and C, $\sim$1600 and $\sim$900 km, respectively). One vial was tested on the day of arrival, *i.e.*, 1 day on dry ice (D1).

Table 1
Experimental design and nomenclature for the single-center study evaluating transport and storage conditions for cryopreserved HPC on dry ice with or without subsequent storage in LN.

Day 0	Day 1	Day 2	Day 3	Day 4
4 vials in LN (LN controls); 1 vial tested on days 1, 2, 3, 4	**L1** in LN only	**L2** in LN only	**L3** in LN only	**L4** in LN only
4 vials placed on dry ice on day 0; 1 vial tested on days 1, 2, 3, 4	**D1** 1 day on dry ice	**D2** 2 days dry ice	**D3** 3 days dry ice	**D4** 4 days dry ice
3 vials placed on dry ice on day 0; 3 vials returned to LN on day 1; 1 vial tested on days 2, 3, 4		**D1 + L1** 1 day on dry ice + 1 day in LN	**D1 + L2** 1 day on dry ice + 2 days in LN	**D1 + L3** 1 day on dry ice + 3 days in LN

Eleven experiments from 10 cryopreserved HPC samples were set up as shown, and $vCD34^+$ HPC enumeration was performed on each day for each storage condition. L refers to storage in LN, and D refers to storage on dry ice, with the associated number referring to the days of storage in each medium.

The second vial was transferred to LN upon arrival, then thawed and tested on day 4 (D1 + L3). The central laboratory also performed the same experiments, as well as LN-stored control samples.

All results were collated by the central laboratory. Evaluation of reproducibility was assessed on the criteria of results within ±10% of the median being deemed comparable, based on the coefficient of variation of the single platform method [5].

Multicenter study

After completion of the pilot study, a multicenter study was initiated by inviting Australian centers enrolled in the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) CD34$^+$ Program to take part. Twelve laboratories from five of the six Australian states participated.

A single cryopreserved vial from product HPC-D was delivered to each of these laboratories on dry ice as described in the pilot study. Participants were instructed to perform the vCD34$^+$ assay on day 1 or 3 (after transferring the vial to LN storage) if testing on the day of arrival was not possible. Control samples stored in LN were also tested on day 1 and 3 by the central laboratory. All results were collated by the central laboratory. Evaluation of reproducibility was assessed on the criteria of results within ±10% of the median being deemed comparable.

Statistical analysis

Analysis was performed with SPSS, version 24 (IBM, Chicago, IL). The *t* test for significantly different from null (0) [10] was applied to the absolute counts for each group and also used to assess differences between pairs of means and SD in each day and storage condition. $P < 0.05$ was considered to be significant. Bonferroni's correction [11] was applied for multiple comparisons between the same data. Correlation analysis of multicenter results was performed using R version 3.6.3 (R Core Team). *Z* scores for comparison of multicenter study results were calculated and interpreted as per ISO13528:2015 [12].

Data sharing statement

For original data, please contact d.ma@amr.org.au.

Results

Evaluation of dry ice and LN storage on vCD34$^+$ in a single center

Dry ice and LN transport and storage conditions (Table 1) were simulated at a central laboratory, and the vCD34$^+$ were enumerated (Figure 1). No significant change in vCD34$^+$ counts over time were observed during continuous storage in LN. Storage on dry ice for 1 day resulted in a significant decrease ($P = 0.011$) (Figure 2 and Table 2) in vCD34$^+$ content relative to the LNC mean. After this initial decrease, a stabilization of vCD34$^+$ content was observed upon subsequent storage in LN (D1 versus D1 + L1; D1 versus D1 + L2, and D1 versus D1 + L3; P = NS). Samples stored in dry ice for 3 and 4 days showed a trend to decreased viable CD34 content (Figure 1); however, it did not reach statistical significance. Note that three D4 samples (HSC-B, -F and -G) were unable to be analyzed owing to a technical fault, reducing the power of the D4 subset and requiring interpretative caution. These

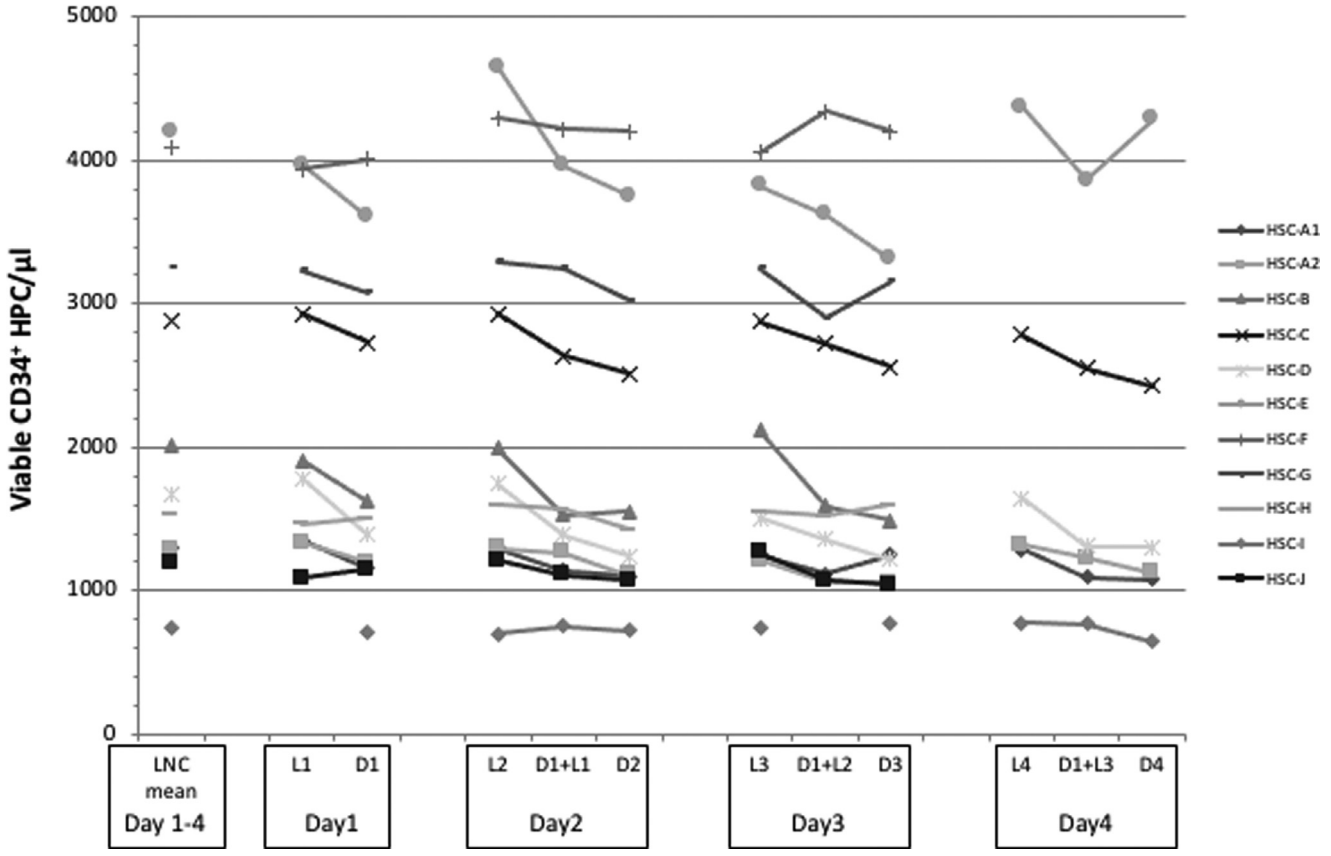

Figure 1. Individual absolute vCD34$^+$ HPC counts from 11 experiments for all storage conditions. vCD34$^+$ HPC enumeration was performed at the central laboratory (Lab A) on 11 HPC samples (representing 10 individual apheresis collections) after various liquid nitrogen (LN) and dry ice (D) storage conditions for ≤4 days. Transfer of cryogenic samples to dry ice consistently resulted in a time-dependent loss of vCD34$^+$ HPCs relative to continuous LN storage. Re-storage in LN after dry ice exposure was associated with higher vCD34$^+$ counts compared with continuous dry ice storage. The LNC mean was calculated from each of the day 1 to 4 LN-stored control samples.

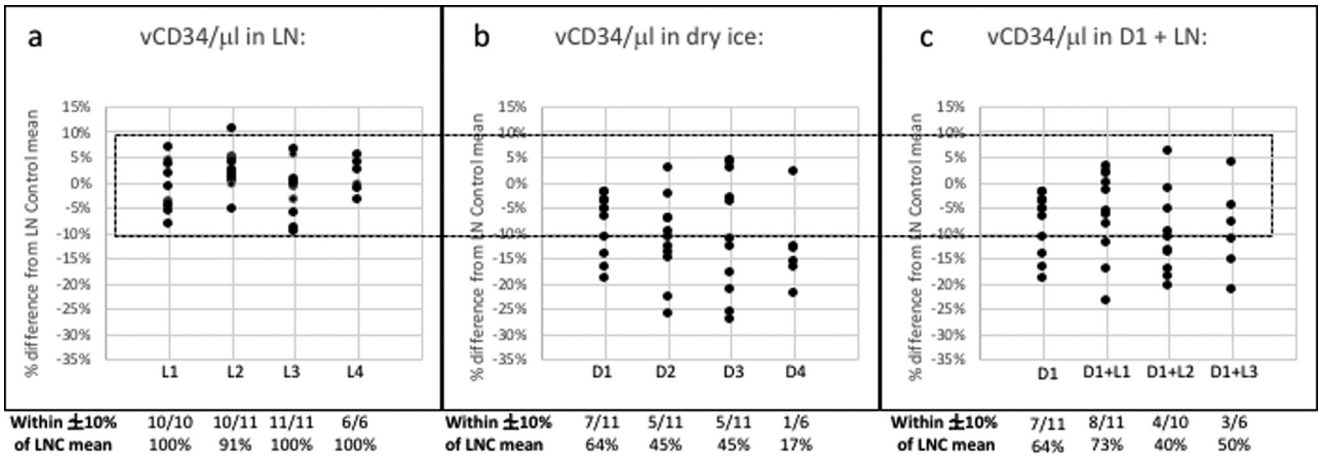

Figure 2. Percentage change in the vCD34$^+$ HPC content relative to the LNC mean. For each storage condition—LN (a), dry ice (b) and dry ice for 1 day then transferred to LN (c)—the vCD34$^+$/μL is shown as percentage difference from the LNC mean for each HPC-A1 to HPC-J. Results within the dotted line rectangle are within ±10% of the LNC mean of each HPC and are enumerated at the base of each panel.

Table 2
Statistical analysis of vCD34$^+$ content in each transport scenario, normalized to the LN control.

	Group A	Group B	Difference in vCD34 (A − B) (%)	P (α, two-tail)
Day 1	L1	D1	7	0.011*
Day 2	L2	D1 + L1	8.4	0.008*
	L2	D2	13.5	<0.001*
	D1 + L1	D2	5.1	0.168
	D1 + L1	D1	1.9	0.167
Day 3	L3	D1 + L2	12.2	<0.001*
	L3	D3	11.8	0.006*
	D1 + L2	D3	−0.4	0.939
	D1 + L2	D1	−0.8	0.768
Day 4	L4	D1 + L3	10.5	0.021
	L4	D4	14.1	0.003*
	D1 + L3	D4	3.6	0.478
	D1 + L3	D1	1.3	0.078

Data were allocated into groups A and B based on storage conditions to enable comparison between the different treatments. The difference between pairs of means and SD in each day and each storage condition was tested. The use of $P < 0.05$ to determine statistical significance was changed to $P < 0.017$ under Bonferroni's correction for multiple comparisons using the same data.

* Statistical significance by t test.

combined results suggested that storage and shipping of CRSs on dry ice for 1 day with subsequent storage in LN for up to 3 additional days warranted investigation as an alternative to LN storage and transport.

Pilot Study

A pilot study was performed to evaluate the effect on vCD34$^+$ HPC of shipping two CRSs from central Lab A to each of peripheral Lab B and Lab C on dry ice with subsequent storage in LN. Both Lab B and Lab C reported that the freighted CRSs arrived frozen with excess dry ice at unpacking (transport distances of ~1600 and ~900 km, respectively). For both samples HPC-E and HPC-G (Figure 3a and 3b), Labs A and B provided vCD34$^+$/μL enumerations that were within acceptable limits of the D1 consensus value. Lab C, however, provided vCD34$^+$ results outside the acceptable limits of both the consensus values and the LN control. Lab C's results were internally consistent for HPC-E but inconsistent for HPC-G, suggesting analytical issues rather than problems with sample stability. These findings suggested that overnight shipping of CRSs on dry ice might provide acceptably reproducible vCD34$^+$ results when the assay was performed on arrival day or after further LN storage.

Multicenter study

Findings from the pilot study were further explored in a multicenter study. A single vial of cryopreserved HPC was shipped in dry ice and delivered by next day transport to each of the 12 laboratories (Table 3) with excess dry ice still present at unpacking. The

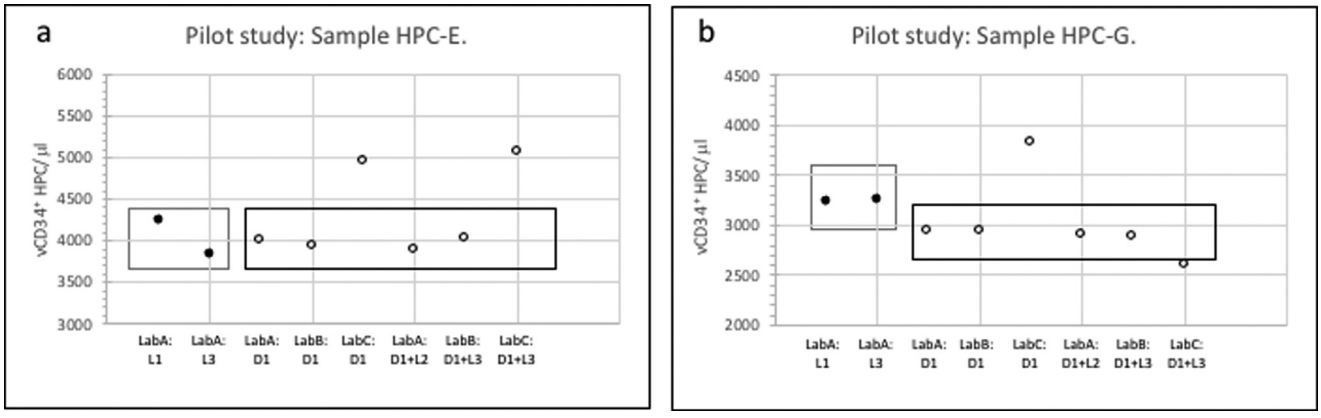

Figure 3. Pilot Study: Comparison of vCD34$^+$ HPC enumerations in three centers after dry ice transport of cryopreserved reference samples. Cryopreserved reference samples, HPC-E (a) and HPC-G (b), were shipped on dry ice from the central laboratory (Lab A) to two other laboratories, Lab B (~1600 km) and Lab C (~900 km). All three facilities performed vCD34$^+$ HPC enumerations at the indicated time points and storage conditions. In addition, LN-stored control samples were analyzed at Lab A on days 1 and 3. Grey outlined rectangles show the median ±10% for samples stored continuously in LN. Black outlined rectangle shows the median ±10% for samples exposed to dry ice for 1 day with or without further storage in LN.

Table 3
A multicenter study evaluating CRS shipping on dry ice and vCD34$^+$ HPC enumeration.

Lab code	Transit distance (km)	Transit time (h)	Storage condition	vCD34$^+$ HPC per μL	*Z* Score
001	0.5	1	D1+L2	1,449	−1.91
002	1	1	D1+L2	**2,028**	0.02
003	7	3.5	D1	1,653	−1.23
004	29	6	D1+L2	**1,912**	−0.37
005	39	6.5	D1	**1,822**	−0.67
006	154	26	D1	**2,016**	−0.02
007	872	23.5	D1+L2	2,431	1.36
008	873	25	D1	**2,128**	0.35
009	918	25	D1	**2,120**	0.32
010	924	22.5	D1+L2	**2,203**	0.60
011	1594	22.5	D1	1,447	−1.91
012	3938	24	D1+L1	**2,065**	0.14

A CRS was shipped to each of 12 different laboratories on dry ice, and vCD34$^+$ HPCs were enumerated on arrival (D1) or after 2 additional days stored in LN (D1 + L2). Bold vCD34$^+$ results represent outcomes within the study consensus range of interlaboratory median ± 10%. There was no relationship between the dry ice transit time, distance couriered or storage condition and the ability to produce a vCD34$^+$ HPC enumeration comparable to other centers. *Z* scores where $|z| < 2$ designates acceptable performance in proficiency testing by interlaboratory comparison.

transportation distances ranged from 0.5 to 4,000 km (median 513 km), with transit times ranging from 1 to 26 h (median 22.5 h).

The 12 centers submitted vCD34$^+$ counts, with six results obtained on day 1 (D1); one result was obtained on day 2 (D1 + L1) and five results were obtained on day 3 (D1 + L2). Overall, 8 of the 12 centers (67%) returned results that were within ±10% of the median and were deemed comparable (Figure 4). There was no significant difference between samples tested on D1 or D1 + L1/L2 ($P = 0.41$). Interestingly, all results fell within the *z* score acceptability limits of $|z| < 2$, with *z* scores ranging from + 1.36 to −1.91. Correlation analysis showed no significant relationship between comparability of vCD34$^+$ counts and (i) sample transit time ($R = 0.67$, $P = 0.07$) or (ii) distance traveled ($R = 0.19$, $P = 0.55$), demonstrating that laboratory outcome was unlikely to be related to sample transport.

Discussion

Flow cytometric enumeration of vCD34$^+$ HPCs is widely used as a quality indicator for assessing the engraftment potential of cryopreserved HPC harvests [13,14]. Such assays on thawed reference vials representative of the cryopreserved HPC products are critical process and quality control points required under both international (*e.g.*, FACT/JACIE [9]) and national (*e.g.*, NPAAC in Australia [15]) clinical facility accreditation standards. However, the data on interlaboratory comparison of vCD34$^+$ HPC enumeration of cryopreserved cells is limited. A recent Australian study [1] found highly variable vCD34$^+$ HPC recoveries in cryopreserved allogeneic HPC procured by national and international centers before the pandemic and highlighted implications for international allogeneic HPC procurement after the sudden pivot from couriering HPCs as a fresh product to transporting as a cryopreserved product owing to COVID-19 travel restrictions. The lack of an EQA for vCD34$^+$ HPC enumeration meant that interlaboratory variability in measurement could not be excluded as a cause. This report illustrates the urgent need to establish EQA programs for vCD34$^+$ HPC enumeration in CRSs, but a major barrier to this is the expense of gold-standard transport of CRS using a LN shipper.

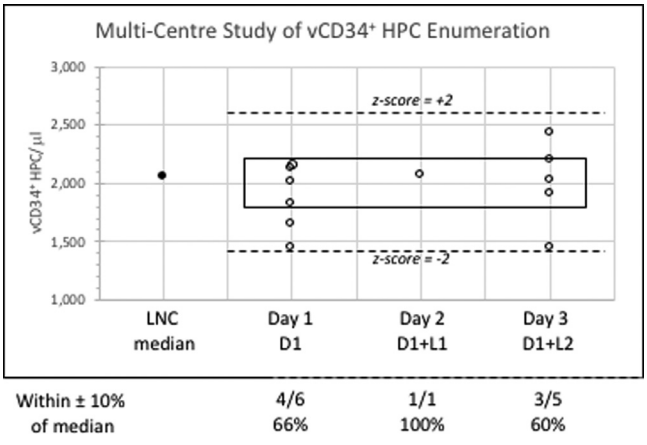

Figure 4. vCD34$^+$ HPC enumeration in the multicenter study of 12 Australian laboratories. Cryopreserved reference samples were shipped on dry ice from the central laboratory to 12 Australian laboratories across distances ranging from 0.5 to 3938 km. vCD34$^+$ HPC enumerations were performed at the indicated time points and storage conditions, with six centers performing the assay on day 1 (D1), one center on day 2 (D1 + L1) and five centers on day 3 (D1 + L2). The solid line rectangle shows the limits of comparability defined as the median ±10%. The *z* score boundaries represent ±2 SD from the median. The number and percentage of participants deemed to provide comparable results at each time point and storage condition is shown at the base of the figure.

Multicenter studies of vCD34$^+$ HPC enumeration using dry shipper transport have been restricted to the setting of cord blood units [16, 17]. Further investigation into this important area for harmonization and standardization has been hampered by the expense of LN dry shipper transport that arises from their limited availability, dangerous goods classification and weight. The estimated cost of dispatch and return of a 30-kg LN shipper by air freight between Australian states is approximately AUD$1500 to $2000 per unit, compared with AUD$70 for a 1.5-kg dry ice–filled insulated container across the same distance one way (≤4000 km). Thus, there is a >20-fold higher cost of LN delivery compared with the dry ice delivery. The capital outlay for LN dry shippers is also considerably more expensive than a reusable expanded foam container. Transport of clinical diagnostic material on dry ice (−78°C) is used widely and is cheaper and easier to handle in many practical senses than LN (−196°) [18].

The present study evaluated the potential for dry ice to be used as a practical and economical transport medium for CRSs in a national multicenter vCD34$^+$ HPC enumeration EQA program. For such a program to be useful and meaningful, it is essential that the reference sample delivered to each participating center has the same analytical properties, such that any resulting variances are attributable to testing methodology rather than the sample. Initial experiments demonstrated that cryopreserved HPC reference vials transferred from cryogenic LN vapor storage to dry ice underwent a significant decrease in vCD34$^+$ HPC content in the first 24 h compared with controls stored continuously in LN. However, when the CRS was returned to LN storage, the vCD34$^+$ HPC content did not show further

significant deterioration. In the context of providing a stable and consistent reference EQA material, we hypothesized that instructing participants to transfer the cryopreserved EQA reference vial to LN after delivery on dry ice would extend the shelf life of the reference material without compromising its analytic comparability with other facilities. This would enable facilities that were unable to test the EQA material on the day of delivery to preserve the sample without invalidating their participation.

The pilot and multicenter studies confirmed that CRS transportation on dry ice provided a consistent reference material to evaluate interlaboratory enumeration of vCD34$^+$ HPCs in an EQA setting. In both cases, the majority of laboratories reported a consensus result that was within ±10% of the median. Of note, in both studies, the laboratories with the longest sample transit times (26 h) and distance travelled (~4000 km) reported vCD34$^+$ HPC counts that were within the limits of consensus. Importantly, and consistent with our single-center evaluation study, re-storage in LN returned a similar proportion of consensus results as samples tested immediately after dry ice delivery, enabling delayed testing of EQA material by recipient centers if required. The multicenter study demonstrated there is some variation in reported vCD34$^+$ HPC enumeration within Australian bone marrow transplant facilities and, consistent with other studies [16, 17], an EQA using cryopreserved HPCs is required to drive inter-facility harmonization and standardization.

The aim of this study was to develop an analytically consistent CRSs for use in an EQA, and as such several limitations arise in interpretation of the pilot and multicenter studies. Instruments, reagents and protocols were not controlled or standardized, leading to potential sources of bias and analytical variability. Despite this, a high level of analytical consistency was achieved, thereby demonstrating the study aim. It is interesting to note that all multicenter study participants recorded a satisfactory z score of $|z|< 2$, which under ISO13528:2015 designates acceptable performance in proficiency testing by interlaboratory comparison [12]. This reinforces the suitability of the proposed CRS dry ice distribution for a vCD34$^+$ HPC enumeration EQA. Further multicenter studies using this methodology will be undertaken to identify critical sources of variability and reduce the observed inconsistency between Australian centers performing vCD34$^+$ HPC enumeration.

Effects comparable to those seen in our study were also noted on the post-thaw function of encapsulated liver spheroids that were similarly cryopreserved to −196°C and transferred to −80°C for 24 h to mimic a storage and distribution scenario [18]. The somewhat surprisingly reduced post-thaw function after short storage at −80°C after LN storage was postulated to be related to the fluidizing of the freeze concentrated matrix between ice crystals on rewarming to −80°C [19]. These findings support the present study that retransferring samples to LN after 24-h storage on dry ice halted the decline in vCD34$^+$ HPC numbers. According to the above hypothesis, this is presumably due to extracellular re-vitrification removing the warming-induced damaging physical changes, and thereby preventing further vCD34$^+$ HPC loss.

Given the critical reliance on vCD34$^+$ HPC content as an indicator of graft quality and patient safety in HPCs derived from cord blood [20] and autologous HPC(A) [14], it is imperative that risks associated with interlaboratory errors and variation in vCD34$^+$ HPC enumeration are minimized through harmonization and standardization underpinned by an EQA. This has heightened relevance in light of the rapid transition to cryopreservation of nationally and internationally procured allogeneic HPCs resulting from COVID-19 disruption to passenger air travel and the identified discordant vCD34$^+$ HPC counts reported by collection and transplant centers. Our study has demonstrated that dry ice transport of cryopreserved HPC for ≤26 h results in the provision of a stable CRS that can enable the commencement of EQA programs for vCD34$^+$ HPC enumeration. This transit time will enable sample distribution across continental distances, while the shelf life can be extended by re-storage in LN at the recipient facility, providing analytic flexibility and convenience.

Declaration of Competing Interest

The authors declare no competing financial interests.

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Authorship Contributions

DM and AC designed and performed the research, analyzed the data, and wrote the manuscript; SR analyzed the data and wrote the manuscript.

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