Developing a National Viable CD34 EQA Program

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Viable CD34+ EQA: Barriers…..

- Ideal Reference Sample would be cryopreserved HPC(A).
- Not available commercially…. Going to need real material.
- Distribution in LN_2 is very expensive and impractical.
  - Dry shippers costly to purchase and to distribute.
  - Easily >$2000 return to any destination involving air freight.

- Dry ice distribution is routinely used to transport diagnostic samples.
- Relatively cheap (~$100-$150), part of existing distribution networks.
- Higher temperature relative to LN_2 (-79°C vs -196C).

Can dry ice distribution provide an analytically consistent Cryopreserved Reference Sample (CRS) for a Viable CD34+ EQA?

Viable CD34+ Enumeration EQA: Feasibility Study

1. Effect of dry ice storage on vCD34+ HPC in cryopreserved samples.
   - Mimic transport scenarios in a single laboratory.
   - Understand impacts of warmer storage temperature on vCD34+ numbers over time.

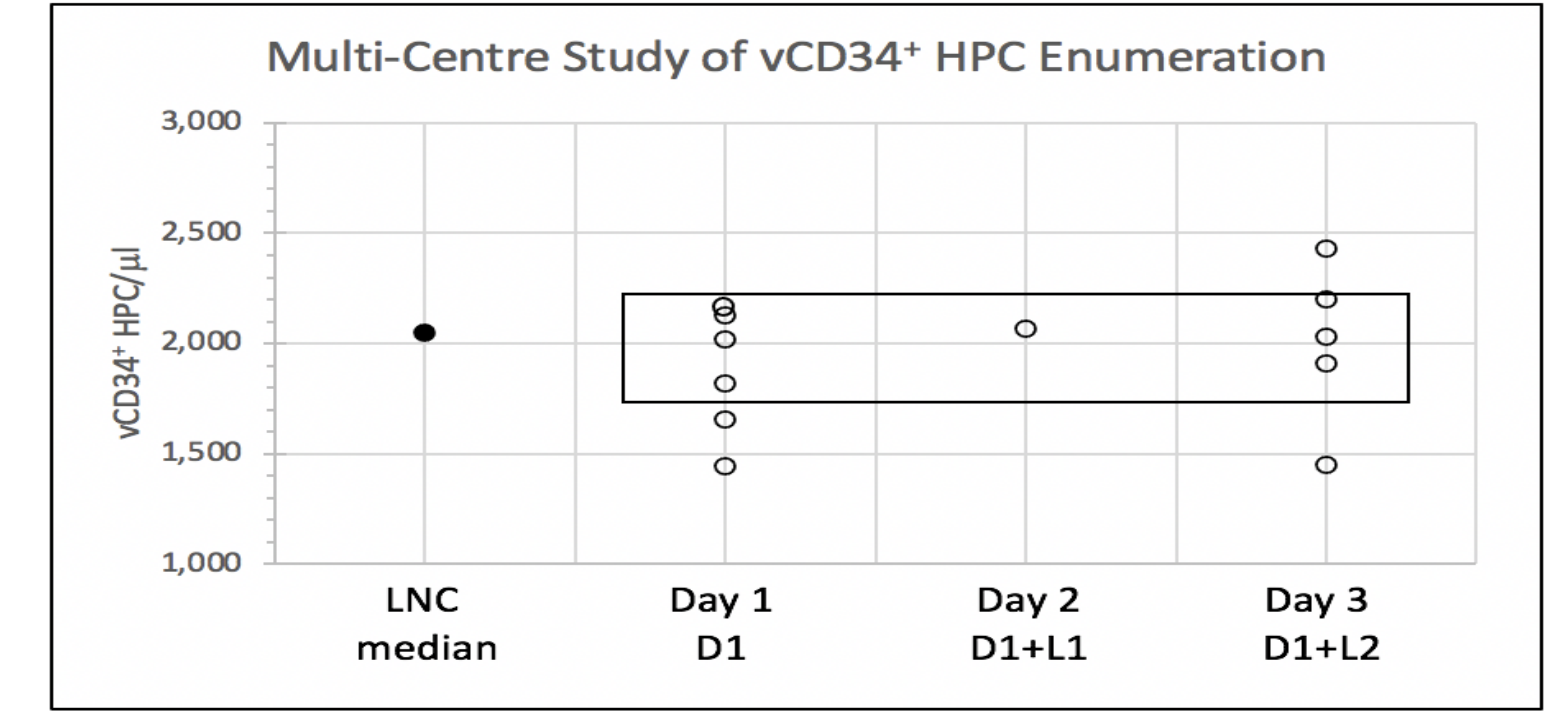
2. Pilot Study: Central Laboratory + 2 interstate Laboratories.
   - Test "real-life" distribution against laboratory findings.
   - Provide "Proof of Principle" for a large scale multicentre study.

3. Multicentre Study: 12 Laboratories across Australia.
   - Mimic an EQA Program distribution.

[Chang, Ragg & Ma (2022) *Cytotherapy* 24: 437-443]

Multicentre Study

- 12 laboratories, 0.5 – 3938km, 1 – 26 hours transit time.
- Enumerate on Day of Arrival (Day 1) or after 2 days LN_2 storage (D1 + L2).
- Comparison Limit set at Median ± 10%



	LNC median	Day 1 D1	Day 2 D1+L1	Day 3 D1+L2
Within ± 10% of median		4/6 66%	1/1 100%	3/5 60%

Distance, Time and Storage Not Related to Comparable Performance.

Lab Code	Transit Distance (km)	Transit time (hours)	Storage condition	vCD34+ HPC per ul
001	0.5	1	D1+L2	1,449
002	1	1	D1+L2	**2,028**
003	7	3.5	D1	1,653
004	29	6	D1+L2	**1,912**
005	39	6.5	D1	**1,822**
006	154	26	D1	**2,016**
007	872	23.5	D1+L2	2,431
008	873	25	D1	**2,128**
009	918	25	D1	**2,120**
010	924	22.5	D1+L2	**2,203**
011	1594	22.5	D1	1,447
012	3938	24	D1+L1	**2,065**

Bold = within (median ±10%) comparison limits

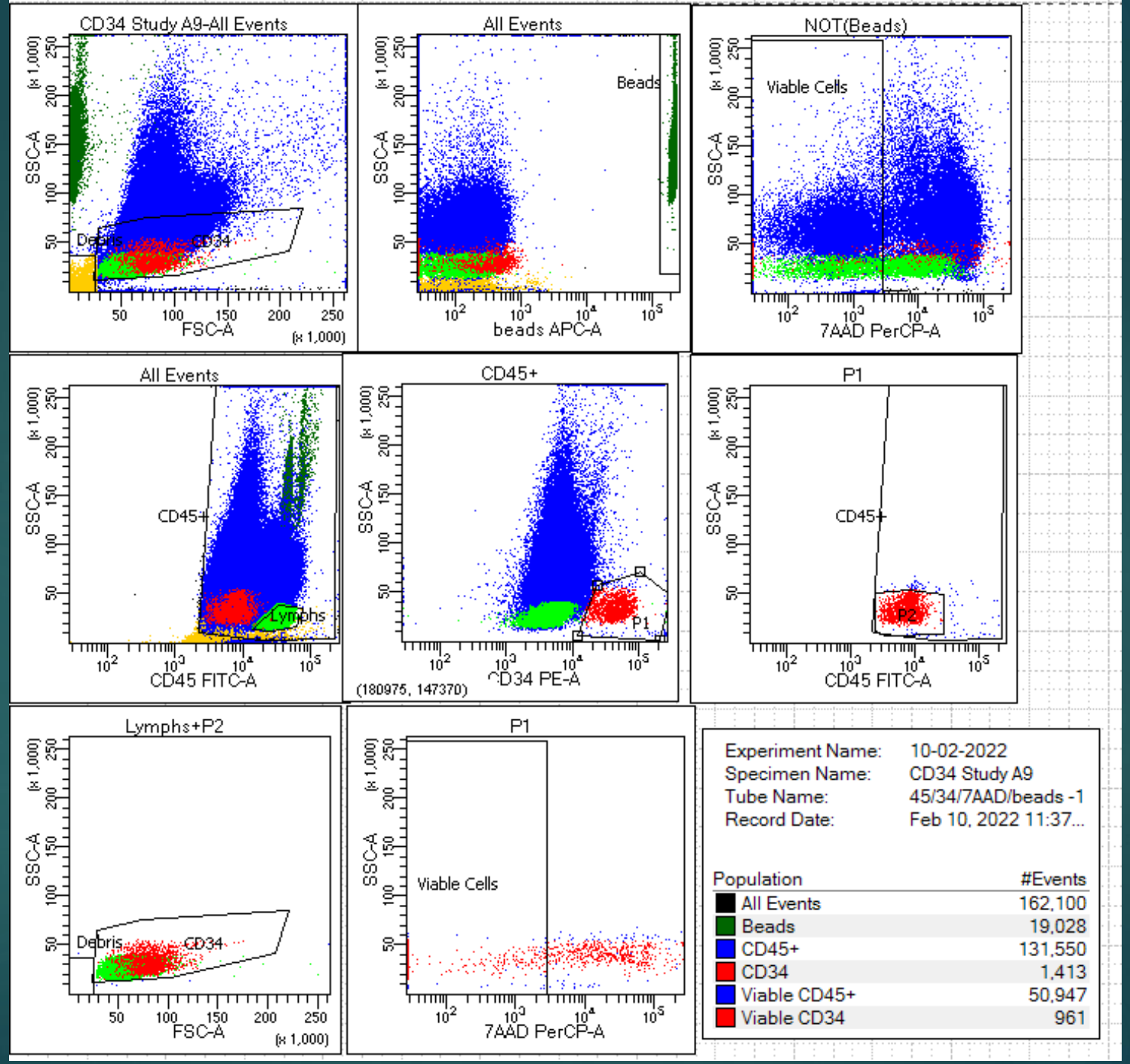
Steps to a National vCD34+ QAP

Not as simple as freezing down some samples, sending them off to your colleagues and getting them to email back the results…..

- Has to run under the auspices of RCPAQAP Inc.
  - They have the QAP experience, we bring the technical expertise.
  - They have NATA accreditation.
  - They will provide sustainability.
- Reliable and easy source of cryopreserved HPC as reference sample.
  - Pilot study used excess HPC(A) from highly mobilised donors.
  - Investigate re-using cryopreserved HPC(A) from deceased patients.
- Demonstrating sample homogeneity and stability to meet NATA accreditation requirements (ISO13528).
- Setting up QAP admin and distribution with RCPAQAP Inc.
- Analysis and interpretation framework (with RCPAQAP Inc)

Discarded HPC(A) Cryoproducts as vCD34+ QAP Material.

- Abundant +++.
- Ethically easy.
- Represents the actual product and form we want to quality assess.
- But, in big bags rather than 0.5ml aliquots.
- Turns out a few of us have been experimenting over the years with thawing, re-packaging and re-cryopreserving HPC(A).
  - Product is thawed, diluted and washed in 5% dextran saline / 2.5% HSA
  - Fresh cryodiluent solution (10% DMSO) prepared and added to cell pellet
  - Aliquotted into multiple (50+) cryovials and cryopreserved in CRF.
  - Has to be tested for homogeneity and stability.

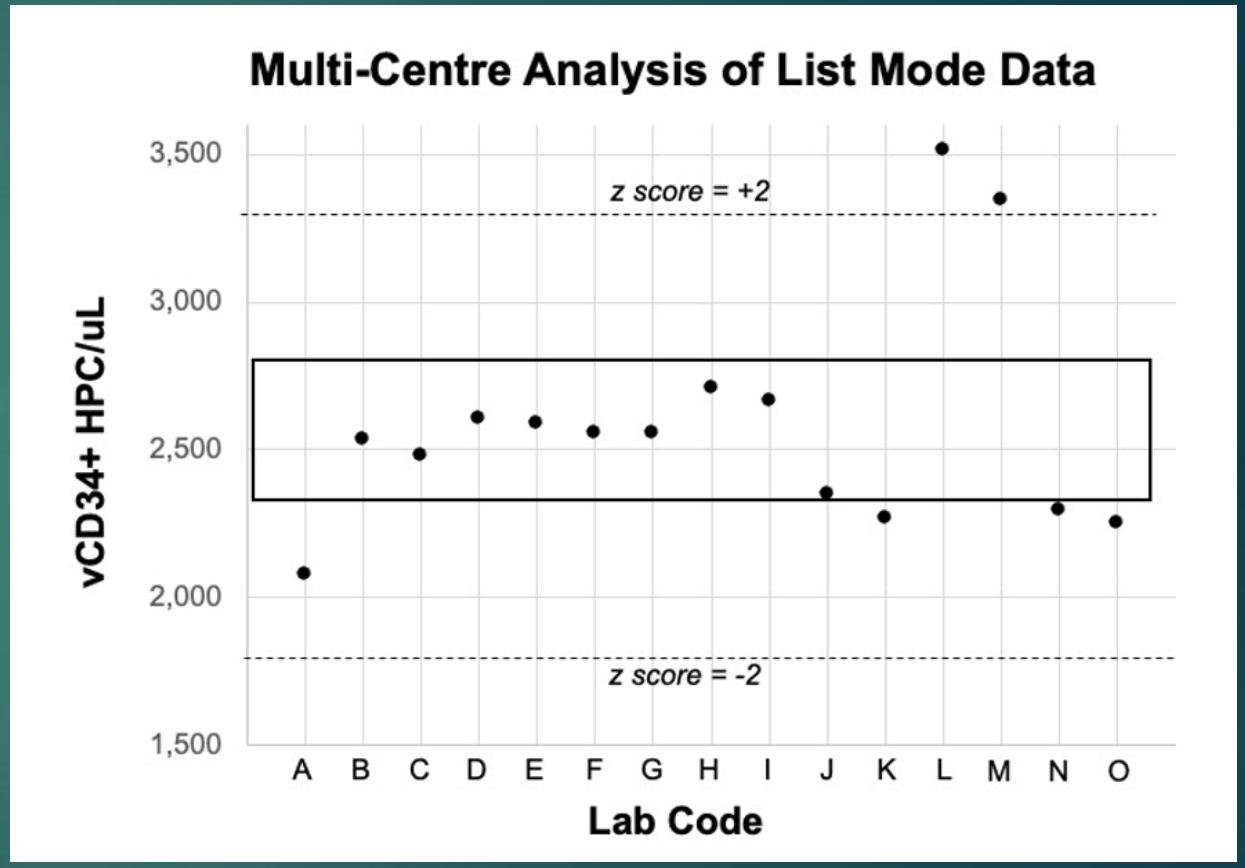

CD34 Study A9-All Events
SSC-A
FSC-A
Debris
CD34
All Events
Beads
beads APC-A
NOT(Beads)
Viable Cells
7AAD PerCP-A
All Events
CD45+
Lymphs
CD45 FITC-A
CD45+
P1
CD34 PE-A
(180975, 147370)
P1
CD45+
P2
CD45 FITC-A
Lymphs+P2
Debris
CD34
FSC-A
P1
Viable Cells
7AAD PerCP-A
Experiment Name: 10-02-2022
Specimen Name: CD34 Study A9
Tube Name: 45/34/7AAD/beads -1
Record Date: Feb 10, 2022 11:37...
Population #Events
All Events 162,100
Beads 19,028
CD45+ 131,550
CD34 1,413
Viable CD45+ 50,947
Viable CD34 961

Homogeneity is Fine.

	A	B	C	D
1	**Cryobag Aliquot**	**T1**	**T2**	**Sample Average (Xt)**
2	A12	1211	1175	1193
3	A24	1152.5	1115.5	1134
4	A13	1269.5	1237.8	1253.65
5	A36	1017.6	1079.8	1048.7
6	A26	1272	1253.1	1262.55
7	A29	1101.7	1102.6	1102.15
8	A7	1281	1247.8	1264.4
9	A33	1227.2	1251.6	1239.4
10	A9	1127	1239.9	1183.45
11	A19	1170	1226.4	1198.2
12				
13	General Average X	1187.95		
14	SD Sample Averages (Sx)	73.1717728		
15	SD Within Sample (Sw)	35.7223459		
16	SD Between Sample (Ss)	68.6736145		
17	Theta pt	237.59		
18	Check value	71.277		
19				

Pre-Analytic vs Analytic factors

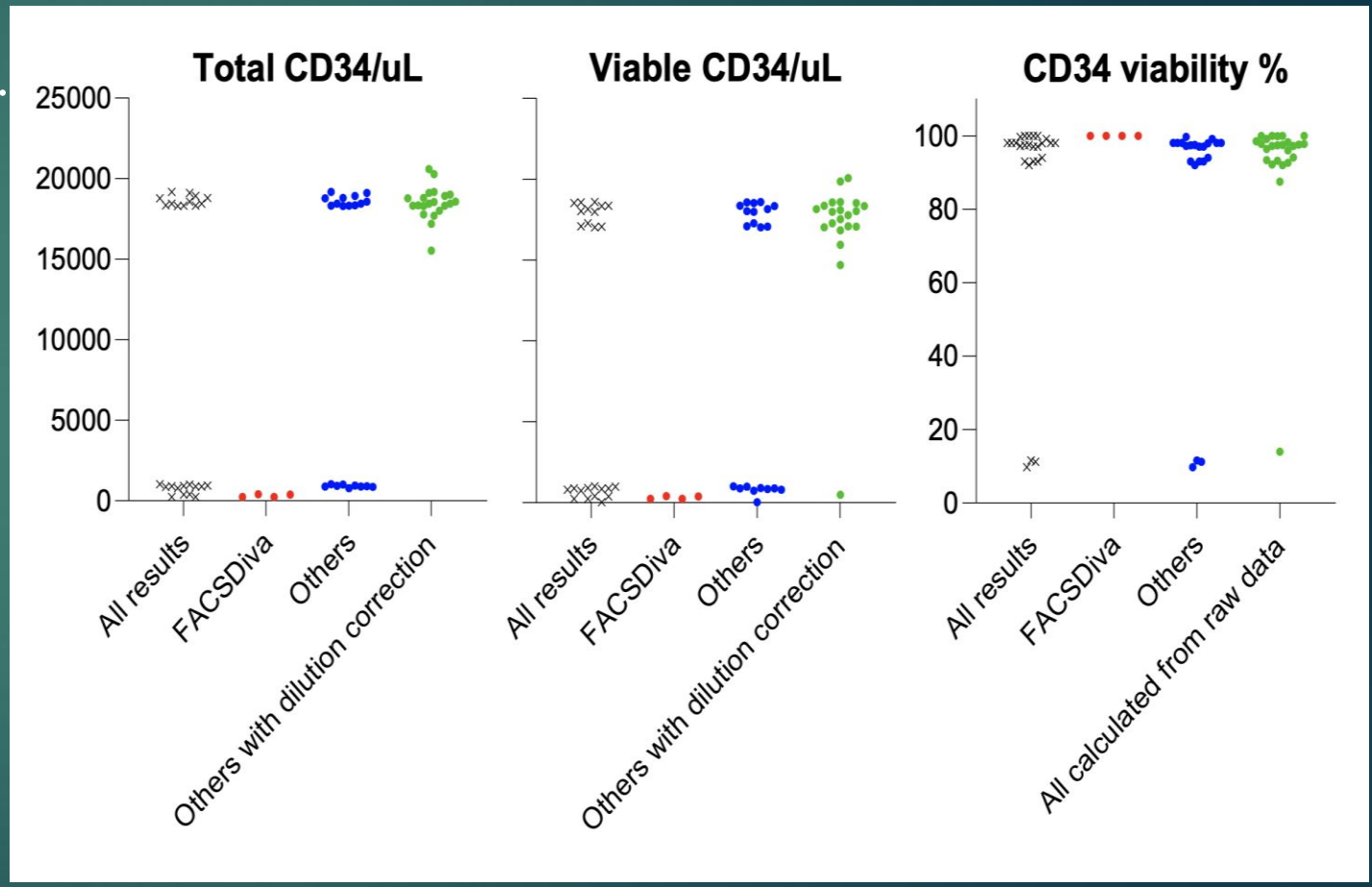
- Result variation can come from sample preparation AND analysis
- A list mode data file sent to labs that responded to an EOI for a trial.
- Still significant variation just from analysis, but 13/15 acceptable.
- Two high outliers were using "dual platform" viable CD34 analysis!
- Review of submitted plots shows highly variable adherence to ISHAGE gating.



Check your viable CD34 gating strategy is correct, especially if you are using a "Modified ISHAGE" template.

More List Mode Data

- Much better return rate than first round.
- Issues with DIVA file – under investigation.
- Labs not incorporating dilution factor into results.
- Results clustering, but still significant variation.
- Viability results indicate placement of 7AAD gate may be inconsistent.
- **Overall: Sufficient consistency to warrant introducing a cryopreserved sample into the QAP.**



Photograph This Slide!

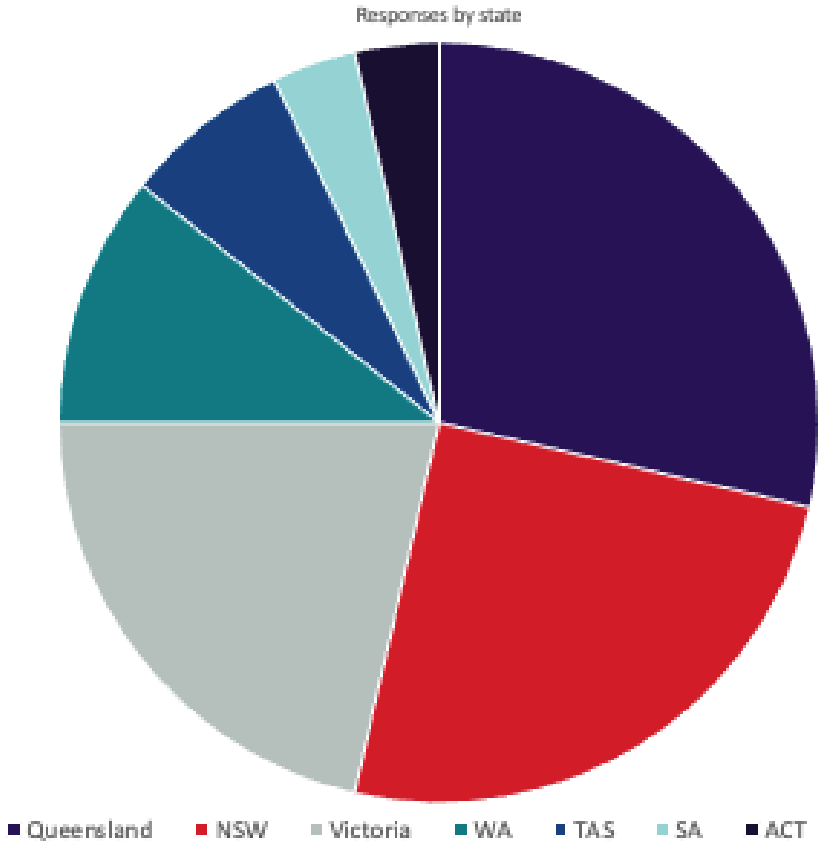
- Rob Sutherland Webinar on CD34 Analysis (incl CD3 and vCD34):
  - https://www.youtube.com/watch?v=50ibWguHqtQ
- Recommended paper on viable CD34 analysis
  - Sutherland et al., *Cytotherapy* (2009) Vol. 11, No. 5, 595–605
- Suggested reading on importance of correct ISHAGE gating for concordant EQA outcomes.
  - Whitby et al., *Cytometry Part B* (2012); 82B: 9–17.

Courtesy of Loriza Khan – RCPAQAP Inc

Expression of Interest for Viable CD34 Feasibility study

- 28 responses received across all states

- When asked how many CD34 tests performed on cryopreserved samples
  - Ranging from 2-3 per month
  - Up to 50-100
  - Median around 10-25 per month



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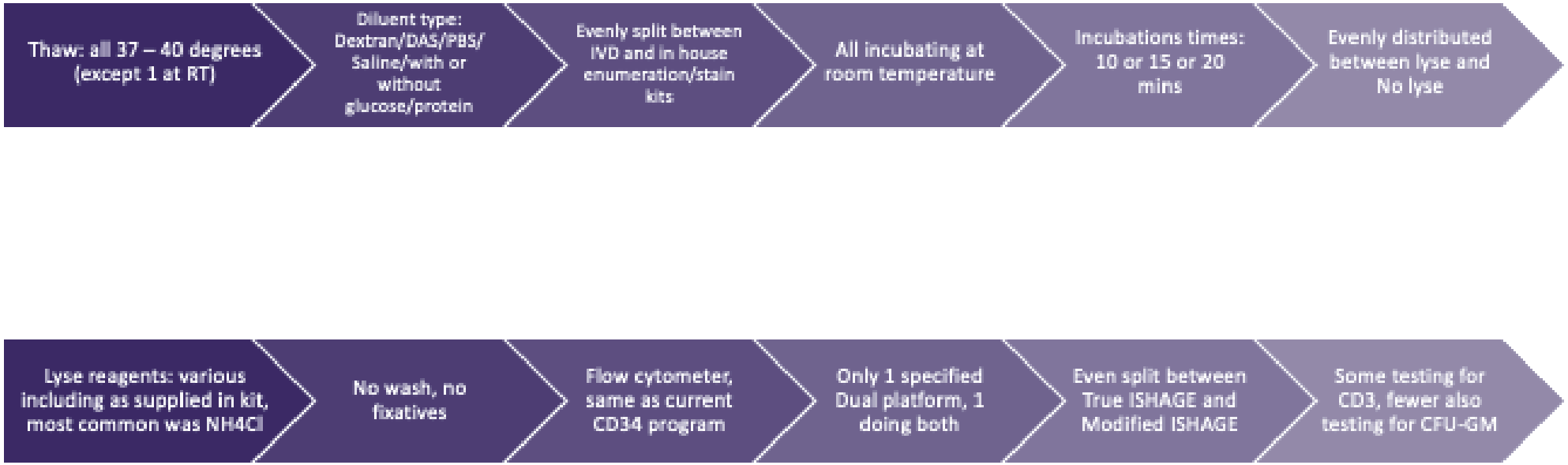
Methodology details from EOI



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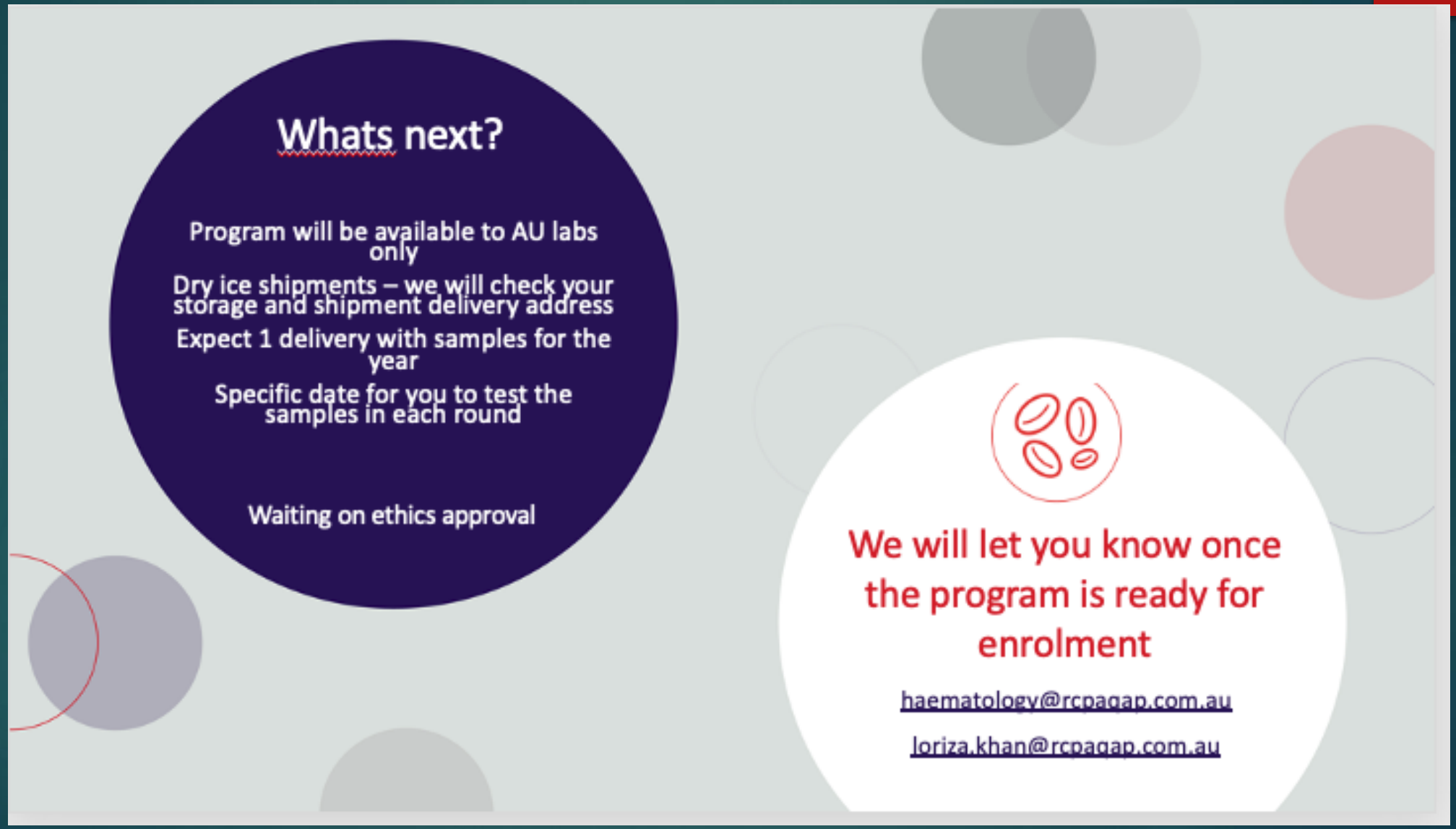

Courtesy of Loriza Khan – RCPAQAP Inc

Viable CD34 available in 2025

Description: Proficiency assessment of viable CD34 cells.

Frequency (No of surveys/year): 3

Cases/samples per survey: 1

Sample Type: Cryopreserved HPC

Sample volume: 0.5mL

Schedule: April, August, November

Measurand/Test: CD3, Total and viable CD45, CD3, CD34

Storage and Handling: Upon receipt store in liquid nitrogen

Dangerous Goods (UN1845) This survey material will be packed in compliance with IATA PI.954 for Dry Ice, UN 1845

Additional Comments: Samples are dispatched on dry Ice. Participants will need to store samples in liquid nitrogen. Restricted to Australia only. 3 surveys per year (2 surveys with 1 sample, 1 survey with dot plot analysis)