Report: 39th IVRN PBMC cryopreservation QAP, Nov 2023

Executive Summary

The 39th IVRN QAP exercise took place on 14th Nov 2023, and laboratory assessment of returned PBMC specimens was completed in Dec 2023. The primary outcomes of this QAP round are:

- A high standard of performance in the 39th QAP round.
- New laboratories (Lab Y & Z) joined the QAP; Lab J did not participate in the round.
- 12 of 13 labs demonstrated efficient PBMC fractionation recovery;
- All 13 labs provided at least one PBMC sample with post-thaw recovery >75%; the recovery standard was met in 30 of 38 PBMC samples tested in this assessment;
- Good quality PBMC: very high viability, and acceptable function results (the response to the CEFX peptide pool is donor dependent);
- 12 of 13 participating laboratories passed this QA round, and 12 labs are currently certified as "Proficient in PBMC Fractionation and Cryopreservation" by the IVRN.

PBMC fractionation recovery

The total PBMC content in blood samples provided by IVRN (approx. 14.5ml per 15ml tube) was calculated from FBCs performed on fresh blood and by participating labs the following day:

PBMC = (lymphocytes + monocytes) x 10^6/ml x 29ml (Table 1).

Table 1. Average PBMC (lymphs+monos) /ml IVRN blood samples: FBC performed fresh and on the day of processing at the labs indicated below.

Laboratory	HIPO (x10^6ml)	HINE (x10^6/ml)	cell counter
fresh blood	2.37	2.18	Coulter DXH500
lab B	2.45	2.25	Sysmex XN20
lab K	2.53	2.21	Sysmex XN10
lab O	2.1	1.9	CellDyn Emerald 22
lab P	2.32	2.11	Coulter DxH500
lab R	2.3	2.0	CellDyn Emerald 22
lab U	2.41	2.25	Abbott Alinity
lab X	2.2	2.4	CellDyn Emerald 22
lab Y	1.9	1.78	Advia 2120
lab Z	2.4	2.1	Sysmex XN-1000
mean PBMC /ml	2.298	2.018	
total PBMC /29ml	66.6	58.5	

Fractionation recovery for IVRN blood samples was calculated as the PBMC count divided by the mean whole blood PBMC counts reported in Table 1. The minimum fractionation recovery standard is 30% of whole blood PBMC. The fractionation recovery for local controls was based on the reported PBMC content, or >1 x 10^6 PBMC per 1ml blood if an FBC was not performed. The mean fractionation recovery from all specimens received was 61.3%, which is at the upper level of recovery expected from careful Ficoll centrifugation (40-60%).

The FBC performed on fresh IVRN blood samples, which is shared with all labs the day before the QAP exercise, can be used as a reference to indicate if the fractionated PBMC count obtained at each lab is within the expected range, or should be repeated/investigated.

Assessment procedures

Thawing and assessment for this QAP round was performed on two consecutive days, including repeat thaws where required. PBMC were thawed and washed in groups of four specimens, resuspended in 5ml, and a 250µl aliquot is counted on a Coulter DxH500 analyser. The PBMC concentration was adjusted to 1 x 10^6/ml, and 100 µl added to prepared antigens (100 µl /well) in pre-coated IFNγ ELISPOT plates, and incubated overnight before development. Manual Trypan Blue viability assessments were performed on the residual 250µl PBMC aliquots, and the manual counts were reported for post-thaw recovery results.

Post-thaw PBMC viability and recovery

Thawed PBMC recovery in this QA round continued the trend toward improved results seen in the previous round, and are now at the highest level observed during the previous 10 QA rounds (Figure 1, Table 2). The quality of thawed PBMC was excellent, with very few non-viable cells observed manually via haemocytometer.

The analysis of recoveries (Figure 2) demonstrates a reciprocal association between low apparent fractionation recovery and excessively high post-thaw recovery, as seen in Lab Y samples, which were likely the result of cell counting errors. The expected efficiency of PBMC recovery from Ficoll purification is approximately 60%. Therefore, a fractionation recovery that is substantially lower than expectation could be due to cell losses in the fractionation process, or in the case of these results from Lab Y, an error in cell counting, which resulted in far more cells added to the vials than reported, and hence the thawed recovery level greater than the defined range.

Overall, absolute recovery represents the real recovery of viable PBMC from the cryopreservation process (Figure 2C), which was very high in QAP round (average 57.2%). However, individual specimens failed if either fractionation recovery or thawed recovery were out-of-range.

Functional analysis

PBMC function in this QA round confirmed that PBMC were of high quality (Figure 3). Background spots were low for all PBMC samples. The response to the CEFX 32-peptide pool was low/undetectable in both IVRN donors, whereas responses from local donors varied across a wide range, confirming immunogenicity of this peptide pool. All PBMS samples responded maximally to polyclonal stimulation with PMA + ionomycin.

Certification status of participating laboratories after the 38th QA round

Twelve of the 13 labs that participated in the 39th QA round provided at least one PBMC specimen that passed all quality standards, and therefore passed this QA round (Table 3). Twelve labs are currently certified by the IVRN as “Proficient in PBMC Fractionation and Cryopreservation”.

Thanks for your ongoing participation in the IVRN PBMC processing QAP. To maintain a high level of proficiency, the IVRN recommends that in the absence of routine PBMC cryopreservation work between QAp rounds, or if new members join your group, please allow time for participating scientists to practice and self-assess performance between QAP rounds. All are encouraged to discuss any methods or performance issues with the QAP coordinator.

39th IVRN QAP report was produced by Dr Wayne Dyer, on behalf of the IVRN Executive.

Table 2. 39th IVRN PBMC Cryopreservation QA Round: PBMC Fractionation Recovery, Viability, Viable Recovery and Function.

									lab data	QAP coordinator data				PBMC function (ELISPOT)						
lab code	donor category	sample date	blood vol	blood PBMC	cells/vial (million)	No. vials	total recovered	[1] fractionation recovery (%)	PMNs in PBMC (%)	thawed PBMCx10^6	[3] post thaw recovery (%)	[6] absolute recovery (%	[2] viability %	**control** spots/well	**net spots/10^6 PBMC** CEF	PMA/Iono	[1] Adequate fractionation	Adequate viability/recovery	[4] Adequate function	[5] Overall result
B	HIV-pos	13/11/2023	29	66.6	10.41	3	31.2	46.9	17.7	8.219	79.0%	37.0	90.7%	3	<20	>5000	yes	yes	yes	
	HIV neg	13/11/2023	29	58.5	11.08	2	22.2	37.9	32.0	10.185	91.9%	34.8	95.1%	7	<10	>5000	yes	yes	yes	pass
	local donor	14/11/2023	16	40.3	10.4	3	31.2	77.4	1.7	9.135	87.8%	68.0	96.7%	3	50	>5000	yes	yes	yes	
E	HIV-pos	13/11/2023	29	66.6	14.2	3	42.6	64.0	NA	10.185	71.7%	45.9	97.0%	3	0	>5000	yes	no	yes	
	HIV neg	13/11/2023	29	58.5	12.8	3	38.4	65.6	NA	7.350	57.4%	37.7	92.1%	4	0	>5000	yes	no	yes	pass
	local donor	14/11/2023	27	64.8	13.2	3	39.6	61.1	NA	12.600	95.5%	58.3	97.6%	1	<20	>5000	yes	yes	yes	
F	HIV-pos	13/11/2023	29	66.6	10	4	40.0	60.1	3.0	10.920	109.2%	65.6	96.3%	3	0	>5000	yes	yes	yes	
	HIV neg	13/11/2023	29	58.5	10	4	40.0	68.4	8.1	8.925	89.3%	61.0	95.5%	4	<10	>5000	yes	yes	yes	pass
	local donor	14/11/2023	27	49.1	10	2	20.0	40.7	1.7	7.455	74.6%	30.4	92.2%	4	0	>5000	yes	no	yes	
J	HIV-pos																			
	HIV neg																			
	local donor																			
K	HIV-pos	13/11/2023	29	66.6	7.9	6	47.4	71.2	4.1	7.035	89.1%	63.4	97.1%	3	0	>5000	yes	yes	yes	
	HIV neg	13/11/2023	29	58.5	6.8	6	40.8	69.7	10.1	6.720	98.8%	68.9	91.4%	2	<10	>5000	yes	yes	yes	pass
	local donor	14/11/2023	29	62.1	6.48	6	38.9	62.6	3.9	5.985	92.4%	57.8	93.4%	12	260	>5000	yes	yes	yes	
O	HIV-pos	13/11/2023	29	66.6	9	6	54.0	81.1	3.0	8.925	99.2%	80.4	97.7%	1	<10	>5000	yes	yes	yes	
	HIV neg	13/11/2023	29	58.5	9	5	45.0	76.9	2.7	9.975	110.8%	85.3	96.9%	3	0	>5000	yes	yes	yes	pass
	local donor	14/11/2023	30	66	10	6	60.0	90.9	0.9	11.025	110.3%	100.2	97.2%	1	0	>5000	yes	yes	yes	
P	HIV-pos	13/11/2023	29	66.6	8.47	5	42.4	63.6	1.1	7.035	83.1%	52.8	97.1%	0	0	>5000	yes	yes	yes	
	HIV neg	13/11/2023	29	58.5	7.77	5	38.9	66.4	3.1	4.935	63.5%	42.2	97.9%	1	<20	>5000	yes	no	yes	pass
	local donor	14/11/2023	15	41.9	9.5	3	28.5	68.0	1.5	8.190	86.2%	58.6	85.9%	0	0	>5000	yes	yes	yes	
R	HIV-pos	13/11/2023	29	66.6	7.97	5	39.9	59.8		8.925	112.0%	67.0	98.5%	3	0	>5000	yes	yes	yes	
	HIV neg	13/11/2023	29	58.5	6.31	4	25.2	43.1		6.930	109.8%	47.4	95.5%	6	0	>5000	yes	yes	yes	pass
	local donor	14/11/2023	30	69	7.65	4	30.6	44.3		6.720	87.8%	39.0		2	90	>5000	yes	yes	yes	
T	HIV-pos	13/11/2023	29	66.6	9.12	5	45.6	68.5	NA	8.925	97.9%	67.0	95.5%	0	<10	>5000	yes	yes	yes	
	HIV neg	13/11/2023	29	58.5	8.66	5	43.3	74.0	NA	3.780	43.6%	32.3	94.7%	0	<10	>5000	yes	no	yes	pass
	local donor	14/11/2023	32	NA	9.28	8	74.2	OK	NA	8.505	91.6%	NA	96.4%	2	0	>5000	yes	yes	yes	
U	HIV-pos	13/11/2023	29	66.6	7.54	6	45.2	67.9	4.7	8.400	111.4%	75.7	94.1%	1	<20	>5000	yes	yes	yes	
	HIV neg	13/11/2023	29	58.5	7.34	4	29.4	50.2	3.2	5.880	80.1%	40.2	93.3%	6	0	>5000	yes	yes	yes	pass
	local donor	14/11/2023	29	51.3	7.62	4	30.5	59.4	1.2	7.665	100.6%	59.8	97.3%	6	110	>5000	yes	yes	yes	
W	HIV-pos	13/11/2023	29	66.6	8.5	4	34.0	51.1	NA	8.505	100.1%	51.1	97.6%	0	<10	>5000	yes	yes	yes	
	HIV neg	13/11/2023	29	58.5	8.17	4	32.7	55.9	NA	6.825	83.5%	46.7	98.5%	1	0	>5000	yes	yes	yes	pass
	local donor	14/11/2023	18	NA	6.83	3	20.5	OK	NA	6.300	92.2%	NA	95.2%	1	880	>5000	yes	yes	yes	
X	HIV-pos	13/11/2023	29	66.6	9.5	6	57.0	85.6	1.3	9.555	100.6%	86.1	97.8%	1	0	>5000	yes	yes	yes	
	HIV neg	13/11/2023	29	58.5	9.4	5	47.0	80.3	1.8	7.770	82.7%	66.4	97.4%	2	0	>5000	yes	yes	yes	pass
	local donor	14/11/2023	30	105	9.75	8	78.0	74.3	0.1	9.555	98.0%	72.8	96.8%	3	290	>5000	yes	yes	yes	
Y	HIV-pos	13/11/2023	29	66.6	5	4	20.0	30.0	4.2	6.300	126.0%	37.8	98.4%	5	0	>5000	yes	no	yes	
	HIV neg	13/11/2023	29	58.5	5	2	10.0	17.1	11.9	8.610	172.2%	29.4	93.2%	6	<20	>5000	no	no	yes	fail
	local donor	14/11/2023																		
Z	HIV-pos	13/11/2023	29	66.6	10.2	4	40.8	61.3	NA	11.660	114.3%	70.0	94.9%	0	0	>5000	yes	yes	yes	
	HIV neg	13/11/2023	29	58.5	8.85	4	35.4	60.5	NA	11.130	125.8%	76.1	94.6%	3	0	>5000	yes	no	yes	pass
	local donor	14/11/2023	16	68.6	8.88	4	35.5	51.8	NA	8.055	90.7%	47.0	89.5%	1	1180	>5000	yes	yes	yes	

Notes: **(1) Assessment criteria 1:** fractionation recovery >30% of available PBMC in whole blood samples, or >1x10^6 PBMC/ml blood if local donor FBC not available.

(2) Assessment criteria 2: Viability >80%, determined by Trypan Blue exclusion (haemacytometer).

(3) Assessment criteria 3: Recovery of viable cells: >75% and <125% of stated vial contents.

(4) Assessment criteria 4: ELISPOT IFNg response (HIV+ & neg, respectively): PMA/Ionomycin: >5000/10^6 PBMC; CEF (mean - 2SD) ≥ 0 SFC/10^6 PBMC; control spots (mean +2SD) ≤ 5 & 8 spots/well.

(5) Adequate results in all 4 criteria from at least one specimen (IVRN or local donor) is required to pass the QAP round. Results outside the assessment criteria.

(6) Absolute recovery = total cells thawed x total number of vials produced / total PBMC in whole blood sample.

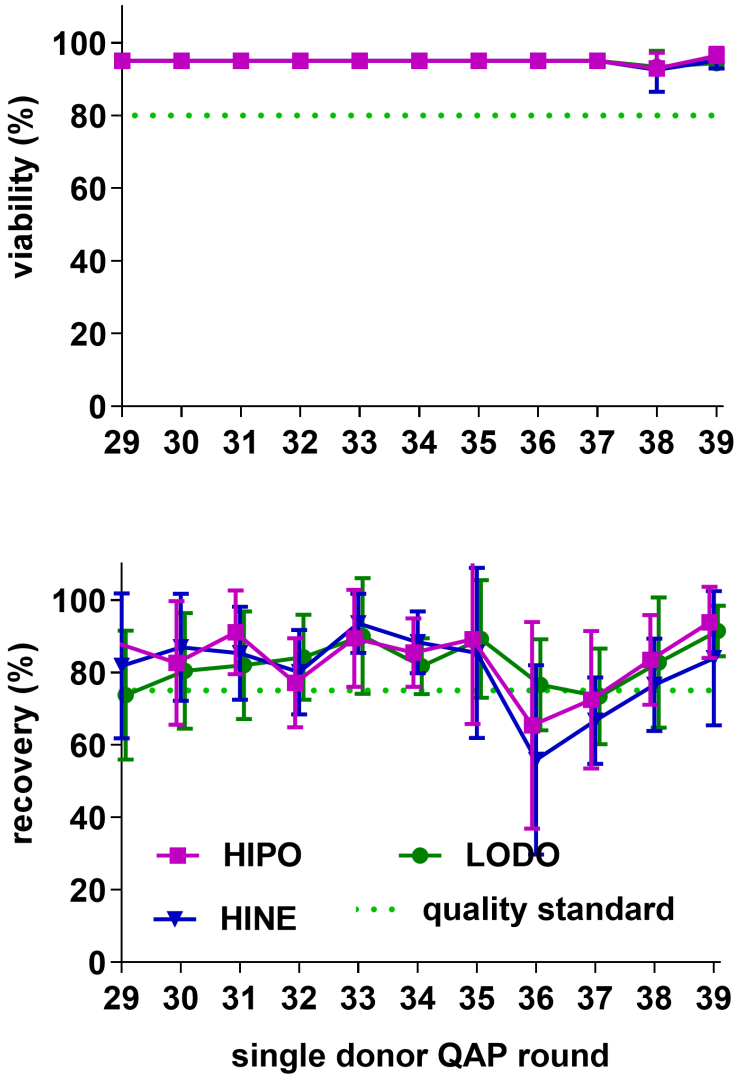

Figure 1. Viability and post thaw recovery compared with the 10 previous QA rounds.
Mean and standard deviation; maximum post-thaw recovery was defined as 100%.

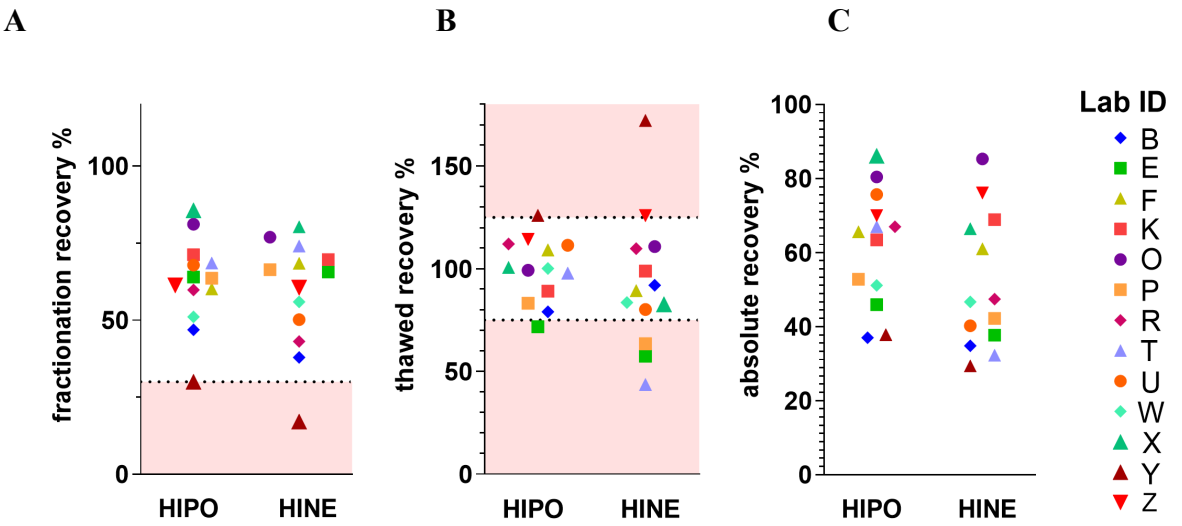

Figure 2. Comparison of relative vs. absolute recovery of PBMC: (A) lab reported fractionation recovery; (B) thawed PBMC recovery relative to vial contents, and (C) absolute recovery (thawed PBMC x total number of vials)/(whole blood PBMC count). Shaded areas in panels A and B define data outside the QA specifications.

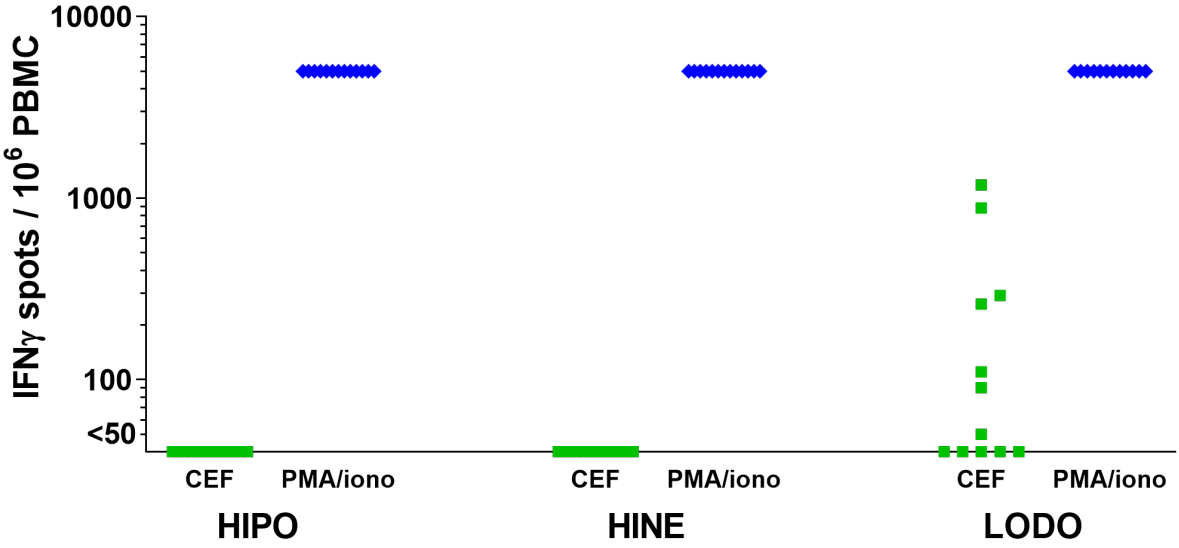

Figure 3. PBMC function results determined by IFN-γ ELISPOT. Antigen-specific responses were determined by stimulation and overnight culture with the CEFX peptide pool, and maximal cytokine release with PMA + ionomycin.

Table 3. Current certification status of Tier 1 labs.

lab code	Adequately performance over the previous QAP exercises? (all 4 quality standards met in at least one PBMC specimen)				current status
	36th QAP	37th QAP	38th QAP	39th QAP	(passed 2 of 3 QAP rounds)
B	fail	fail	fail	pass	**Certified**
E	pass	fail	pass	pass	**Certified**
F	pass	pass	pass	pass	**Certified**
J	NA	pass	fail	NA	**Certified Under Review**
K	fail	pass	pass	pass	**Certified**
O	fail	pass	pass	pass	**Certified**
P	pass	pass	pass	pass	**Certified**
R	fail	fail	pass	pass	**Certified**
T	pass	fail	pass	pass	**Certified**
U	pass	pass	pass	pass	**Certified**
W	pass	fail	pass	pass	**Certified**
X			pass	pass	**Certified**
Y				fail	**not certified**
Z				pass	**Certified**

Notes (extracted from the IVRN Laboratory Performance Policy):

Performance required for ongoing certification as a Tier 1 Laboratory: The performance standards (above) must be attained from at least one PBMC specimen (IVRN single or local donor), from at least 2 out of the past 3 QA rounds. A certificate of satisfactory performance will be issued after each QA round.

Remedial action if a laboratory fails to maintain certification:

- After two consecutive failed attempts at satisfactory performance, the laboratory will be classified as "Certified - Under Review", which recommends that the laboratory continue participation in current clinical trials and cohort studies, but recommendation for involvement in new studies be deferred until evidence of remedial action to improve performance is provided. Laboratory staff will be contacted by the QAP coordinator with the aim of identifying potential causes for the below standard performance, and interventions put in place to achieve the quality standard.
- After three consecutive failed attempts at satisfactory performance, the laboratory will be classified as "Unsatisfactory". In due regard for confidentiality of the status of each laboratory, it is the responsibility of the laboratory that is downgraded to "Unsatisfactory" status to notify the relevant clinical trial sponsor of this change of status. The IVRN will not distribute any details of laboratory performance to a third party. The consequence of this change in status is for negotiation between the laboratory and the clinical trial coordinator/sponsor.
- The IVRN Steering Committee will negotiate a remedial plan with the head of a laboratory that becomes "Unsatisfactory" to assist in improving performance. "Certified Under Review" status will be reinstated upon attainment of a satisfactory result in the subsequent QA round, then "Certified" status after two consecutive pass results. If negotiation and remedial actions are unsuccessful, termination of Tier One laboratory status will be recommended to the IVRN Steering Committee.