Report: 36th IVRN PBMC cryopreservation QA round, Dec 2021

Executive Summary

The 36th IVRN QA exercise took place on 7th Dec 2021, and laboratory assessment of returned PBMC specimens was completed in Dec 2021. The primary outcomes of this QA round are:

- Efficient PBMC fractionation recovery from 9 of 11 labs;
- Acceptable post-thaw recovery from 7 of 11 labs, with poor post-thaw recovery 64% of samples received;
- Good quality PBMC: very high viability and function results;
- 6 of 11 participating laboratories passed this QA round, and 10 labs are currently certified by the IVRN for PBMC cryopreservation.

PBMC fractionation recovery

The total PBMC content in the blood samples provided by IVRN was calculated from FBCs provided by participating labs:

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

The minimum expected fractionation recovery was 30% of whole blood PBMC, or >1 x 10^6 PBMC per 1ml blood from the local donor specimen was if a FBC was not performed. The mean fractionation recovery from all specimens received was 56%, which is within the expected level of recovery from careful Ficoll centrifugation (40-60%). Fractionation recovery, was low in three labs and contributed to the failed QAP outcome in these labs (Table 2).

Table 1. Total PBMC in 29ml whole blood samples: FBC performed fresh and on the day of processing.

Laboratory	HIPO (x10^6/ml)	HINE (x10^6/ml)	cell counter
fresh blood	1.87	2.44	Coulter DXH500
lab B, R	1.73	2.66	Sysmex XN20
lab K	1.67	2.54	CellDyn Emerald
lab O	2.0	2.7	CellDyn Emerald
lab P	1.75	2.61	Coulter DXH500
lab U	1.67	2.59	CellDyn Sapphire
mean	1.764	2.591	
total PBMC in 29ml blood	51.17 x10^6	75. 14x10^6	

Post-thaw PBMC viability and recovery

Viability of thawed PBMC specimens was determined by visual inspection of cells in the presence of trypan blue, confirmed by manual counting if more than a few stained cells were present in a field of view. The quality of thawed PBMC in this QA round was outstanding, with practically no non-viable cells seen in each quadrant of the haemocytometer, and therefore all specimens were rated >95% viable (Figure 1, Table 2). Thawed cell recovery in this QA round was poor compared to previous rounds (Figure 1, Table 2), with 64% of all specimens assessed failing to achieve the 75% recovery standard.

The analysis of recoveries (Figure 2) demonstrates the association between high apparent fractionation recovery and correspondingly low thawed recovery, which is the result of errors (overestimation) in cell counting. Notice that all specimens with a fractionation recovery >75% had post-thaw recovery <75%. Since the expected upper range of PBMC recovery from Ficoll purification is 50-60%, reported fractionation recoveries >60% probably represent overestimation of cell counts. Conversely, underestimation of fractionated cell counts can result in a thawed recovery >100%, as is demonstrated by lab E (green squares). In most cases of overestimation or underestimation, the Absolute Recovery was in the 30-50% range, but individual specimens failed if either fractionation recovery or thawed recovery were out-of-range. The real concern for efficient PBMC preparation is when both fractionation recovery and thawed recovery results are low (eg. Labs B and V), which resulted in a very low Absolute Recovery.

The cumulative trend in viability and post-thaw recovery over the past 10 QAP rounds is shown in Figure 2, and suggests that QAP performance declined in this QA round compared to previous rounds. Understandably, with 12 months since the previous QA round thanks to COVID-associated delays, these results may also reflect possible reduced PBMC fractionation workload at some sites and increased staff turn-over, leading to lapse in proficiency or experience.

Functional analysis

PBMC function in this QA round confirmed that PBMC were of high quality. Immune function was determined by IFNγ ELISPOT assay, measuring the response to the CEF peptide pool (epitopes from CMV, EBV and Influenza), and maximal stimulation from PMA and ionomycin (Figure 3). The response to the CEF peptide pool was uniformly strong in PBMC from the HIV-pos donor, and undetectable in the HIV-neg donor, consistent with previous donations from this volunteer. Individual local donor responses varied from undetectable to strong, confirming immunogenicity of the peptides. All PBMC samples showed maximal stimulation in the presence of PMA and ionomycin (>5000 spots/million PBMC). Specimens from lab K had high background responses in control wells, while high background previously seen in PBMC from Lab F have now substantially improved.

Certification status of participating laboratories after the 35th QA round

All labs achieved uniformly high viability results, 8 of 11 labs achieved sufficient fractionation recovery, 7 of 11 achieved sufficient post-thaw recovery, resulting in only 5 of 11 labs meeting all the quality standards in at least one of the specimens provided, and passing this QA round (Table 3).

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 QA rounds, or if new members join your group, please allow time for participating scientists to practice and self-assess performance between QA rounds. All are encouraged to discuss any methods or performance issues with the QAP coordinator.

36th IVRN QAP report was produced by Dr Wayne Dyer, on behalf of the IVRN Executive.

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% for these mean & SD data.

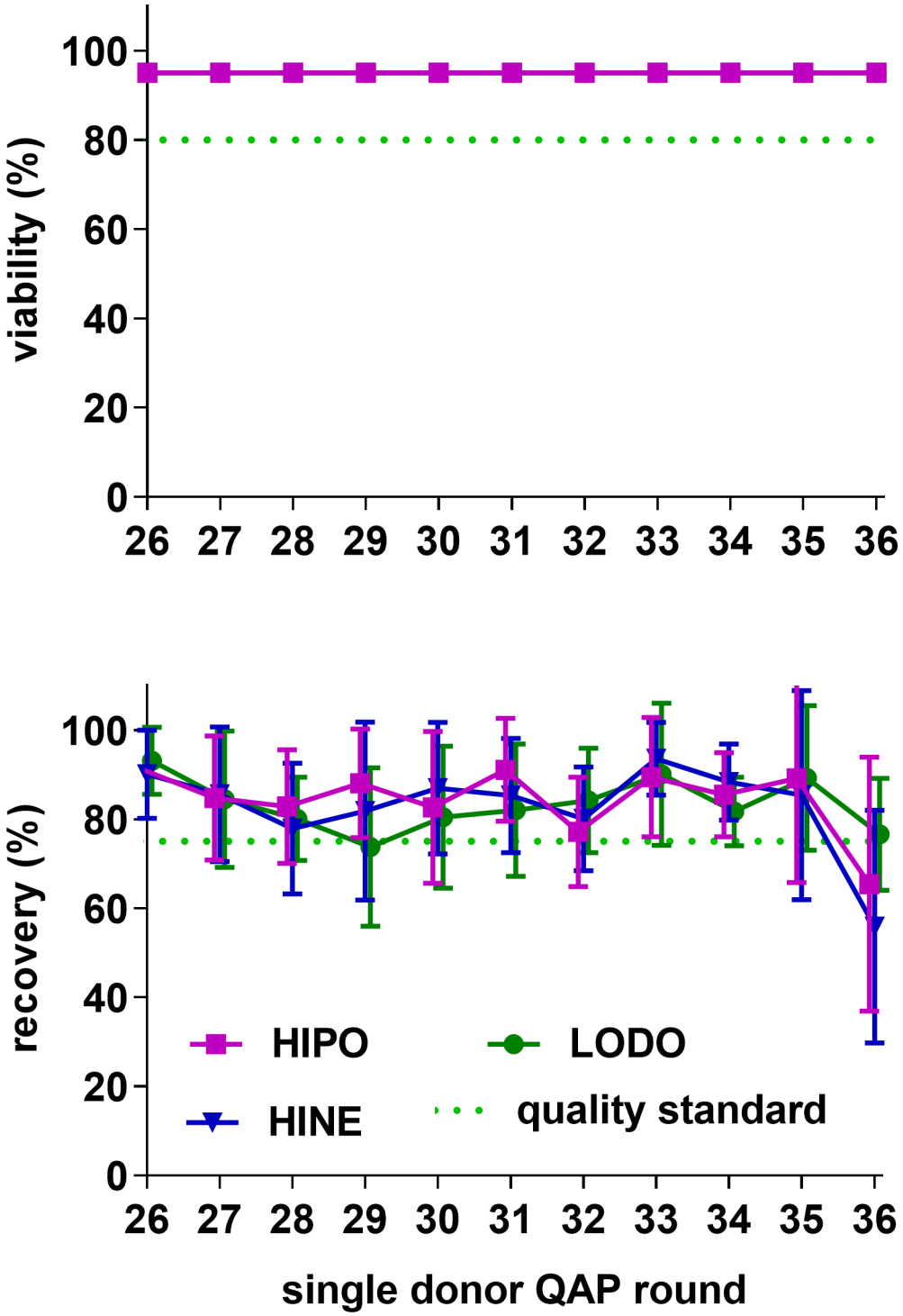

Table 2. 36th IVRN Single Donor QA Round: PBMC Fractionation Recovery, Viability, Viable Recovery and Function.

			lab data						QAP coordinator data				PBMC function (ELISPOT)						
lab code	donor category	sample date	blood vol	cells/vial (million)	No. vials	total recovered	blood PBMC	[1] fractionation recovery (%)	thawed PBMCx10[6]	[3] post thaw recovery (%)	[6] absolute recovery (%	[2] viability %	control spots/well	net spots/10[6] PBMC CEF	net spots/10[6] PBMC PMA/Iono	[1] Adequate fractionation	Adequate viability/recovery	[4] Adequate function	[5] Overall result
B	HIV-pos	6/12/21	29	7.3	1	7.3	51.17	14.3	5.300	72.6	10.4	>95	0	830	>5000	no	no	yes	
	HIV neg	6/12/21	29	8.1	1	8.1	75.14	10.8	5.650	69.8	7.5	>95	2	0	>5000	no	no	yes	fail
	local donor	7/12/21	17	4.5	1	4.5	21.93	20.5	2.700	60.0	12.3	>95	1	20	>5000	no	no	yes	
E	HIV-pos	6/12/21	29	7.3	2	14.6	51.17	28.5	8.200	112.3	32.0	>95	3	2940	>5000	no	yes	yes	
	HIV neg	6/12/21	29	6	3	18	75.14	24.0	6.700	111.7	26.8	>95	1	0	>5000	no	yes	yes	pass
	local donor	7/12/21	30	7.2	4	28.8	66	43.6	6.850	95.1	NA	>95	1	70	>5000	yes	yes	yes	
F	HIV-pos	6/12/21	29	10	3	30	51.17	58.6	9.350	93.5	54.8	>95	3	2720	>5000	yes	yes	yes	
	HIV neg	6/12/21	29																pass
	local donor	7/12/21	27	8	3	24	46.98	51.1	6.150	76.9	39.3	>95	2	10	>5000	yes	yes	yes	
K	HIV-pos	6/12/21	29	6.67	2	13.34	51.17	26.1	5.900	88.5	23.1	>95	65	1820	>5000	no	yes	high control	
	HIV neg	6/12/21	29	7.44	5	37.2	75.14	49.5	5.200	69.9	34.6	>95	11	0	>5000	yes	no	yes	fail
	local donor	7/12/21	27	8.4	5	42	67.77	62.0	6.870	81.8	50.7	>95	187	60	>5000	yes	yes	high control	
O	HIV-pos	6/12/21	29	7	4	28	51.17	54.7	4.200	60.0	32.8	>95	2	2250	>5000	yes	no	yes	
	HIV neg	6/12/21	29	7.43	7	52.01	75.14	69.2	3.600	48.5	33.5	>95	0	0	>5000	yes	no	yes	fail
	local donor	7/12/21	20	8.25	8	66	72	91.7	4.850	58.8	53.9	>95	0	10	>5000	yes	no	yes	
P	HIV-pos	6/12/21	29	6.87	6	41.22	51.17	80.6	5.150	75.0	60.4	>95	2	2470	>5000	yes	yes	yes	
	HIV neg	6/12/21	29	6.72	8	53.76	75.14	71.5	4.350	64.7	46.3	>95	1	10	>5000	yes	no	yes	pass
	local donor	7/12/21	16	6.06	2	12.12	18.88	64.2	4.350	71.8	46.1	>95	34	2130	>5000	yes	no	yes	
R	HIV-pos	6/12/21	29	5.6	6	33.6	51.17	65.7	3.350	59.8	39.3	>95	2	2260	>5000	yes	no	yes	
	HIV neg	6/12/21	29	9.2	8	73.6	75.14	98.0	4.350	47.3	46.3	>95	0	0	>5000	yes	no	yes	fail
	local donor	7/12/21	16	5	2	10	20.64	48.4	3.300	66.0	32.0	>95	0	10	>5000	yes	no	yes	
T	HIV-pos	6/12/21	29	9	5	45	51.17	87.9	3.350	37.2	32.7	>95	1	1490	>5000	yes	no	yes	
	HIV neg	6/12/21	29	9.6	7	67.2	75.14	89.4	4.050	42.2	37.7	>95	1	30	>5000	yes	no	yes	pass
	local donor	7/12/21	15	7.5	3	22.5	NA	OK	6.050	80.7	NA	>95	0	760	>5000	yes	yes	yes	
U	HIV-pos	6/12/21	29	5.69	5	28.45	51.17	55.6	4.950	87.0	48.4	>95	2	2420	>5000	yes	yes	yes	
	HIV neg	6/12/21	29	8.95	5	44.75	75.14	59.6	6.650	74.3	44.3	>95	0	10	>5000	yes	no	yes	pass
	local donor	7/12/21	30	7.39	5	36.95	70.68	52.3	6.900	93.4	48.8	>95	1	0	>5000	yes	yes	yes	
V	HIV-pos	6/12/21	29	5.04	2	10.08	51.17	19.7	0.350	6.9	1.4	>95	x	x	x	no	no	NA	
	HIV neg	6/12/21	29	6.45	2	12.9	75.14	17.2	0.150	2.3	0.4	>95	x	x	x	no	no	NA	fail
W	HIV-pos	6/12/21	29	10	5	50	51.17	97.7	3.900	39.0	38.1	>95	1	1270	>5000	yes	no	yes	
	HIV neg	6/12/21	29	10	6	60	75.14	79.9	4.050	40.5	32.3	>95	1	10	>5000	yes	no	yes	pass
	local donor	7/12/21	18	5	5	25	NA	OK	4.100	82.0	NA	>95	1	3200	>5000	yes	yes	yes	
F	LD SepMate	7/12/21	27	10	3	30	46.98	63.9	7.150	71.5	45.7	>95	1	20	>5000	yes	no	yes	NA
K	LD SepMate	7/12/21	27	8.8	5	44	67.77	64.9	4.750	54.0	35.0	>95	41	270	>5000	yes	no	high control	NA

Notes: **(1) Assessment criteria 1:** fractionation recovery >30% of available PBMC in 30ml whole blood, or >1x106 PBMC/ml blood if local donor FBC not available.

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

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

(4) Assessment criteria 4: ELISPOT (HIV+ & neg; high outliers excluded): PMA/Ionomycin: >5000/10^6 PBMC; CEF (mean - 2SD) >679 & >0/10^6 PBMC; control spots (mean +2SD) <5 & <10 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.

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

Red Results that failed the assessment criteria.

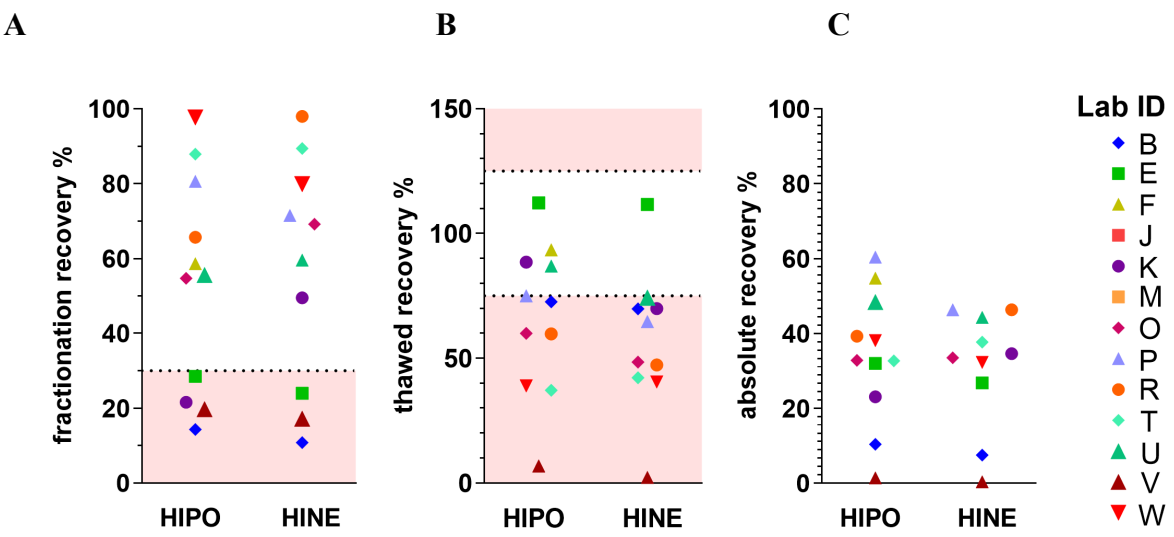

Figure 2. Comparison between relative vs. absolute recovery of PBMC showing (A) post fractionation recovery relative to laboratory cell count; (B) thawed PBMC recovery relative to laboratory cell count, and (C) absolute recovery of PBMC (total thawed PBMC x number of vials) expressed as the % of the mean 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 CEF peptide pool, and maximal cytokine release with PMA + ionomycin.

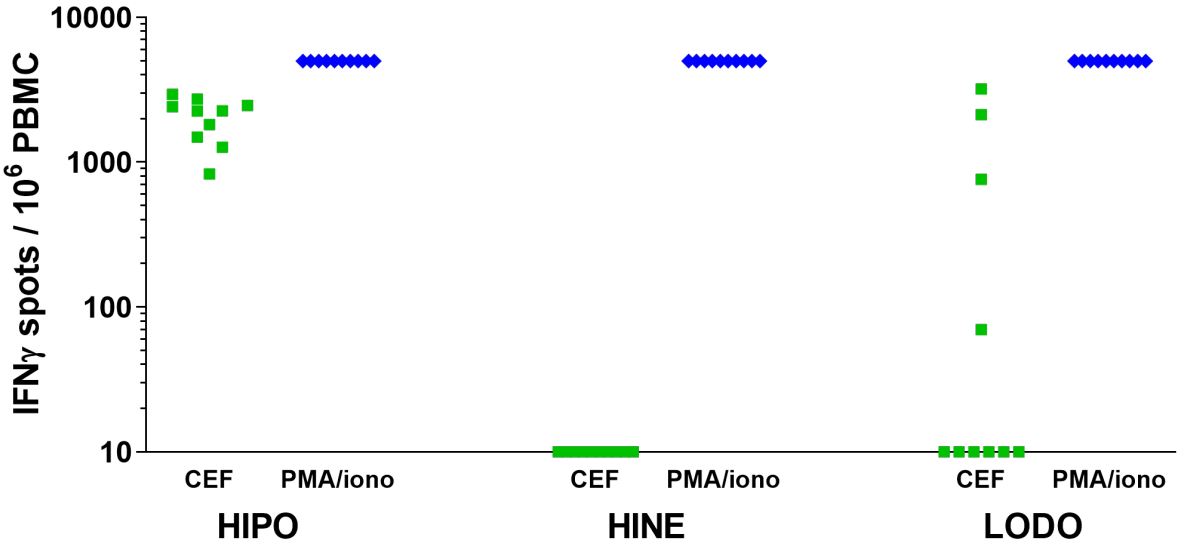

Table 3. Current certification status of Tier 1 labs.

lab code	Adequately performance over the previous QAP rounds? (all 4 quality standards met in at least one PBMC specimen)			current status
	34th round	35th round	36th round	(passed 2 of 3 QAP rounds)
B	pass	fail	fail	**certified under review**
E	pass	pass	pass	**Certified**
F	pass	pass	pass	**Certified**
J	pass	pass	NA	**Certified**
K	pass	pass	fail	**Certified**
M	pass	NA	NA	**not certified**
O	pass	pass	fail	**Certified**
P	pass	pass	pass	**Certified**
R	pass	pass	fail	**Certified**
T	pass	pass	pass	**Certified**
U	pass	pass	pass	**Certified**
V		fail	fail	**not certified**
W		pass	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. Non-participation in a QA round is designated as a failed result. A certificate of satisfactory performance will be issued to each successful laboratory after each QA round.

Remedial action if a laboratory fails to maintain accreditation:

- Upon losing fully "Certified" status, a laboratory will be issued with an "Certified - Under Review" report, which recommends that the laboratory continue participation in current clinical trials and cohort studies, but 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 two 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. If the response is deemed acceptable, "Certified Under Review" status will be reinstated upon attainment of a satisfactory result in the subsequent QA round. If the negotiation is unsuccessful, termination of Tier One laboratory status will be recommended to the IVRN Steering Committee.