Report: 35th IVRN PBMC cryopreservation QA round, Dec 2020

Executive Summary

The 35th IVRN QA exercise took place on 9th Dec 2020, and assessment of returned PBMC specimens was completed in Dec 2020. The primary outcomes of this QA round are:

- Efficient PBMC fractionation;
- Overall improvements in post-thaw recovery;
- Good PBMC function despite low response to CEF peptides from both IVRN donors;
- 10 of 12 participating laboratories passed this QA round, one lab was not available, and 12 labs are currently certified by the IVRN for PBMC cryopreservation.

PBMC fractionation recovery

Fractionation recovery was calculated from the full blood differential counts provided from participating labs (Table 1). The mean fractionation recovery from all specimens was 51%, which is the expected level of recovery from careful Ficoll centrifugation. The minimum accepted fractionation recovery from the local donor specimen was >1 x 10^6 PBMC per 1ml blood if a FBC was not performed. Fractionation recovery, calculated from the average PBMC content of the whole blood specimens (Table 1), was uniformly high, with the exception of lab B (Table 2).

Table 1. Total PBMC in 30ml whole blood samples for 34th QA round, reported from each lab on the day of processing.

Laboratory	HIPO (x10^6/30ml)	HINE (x10^6/30ml)	cell counter
fresh blood	73.1	67.7	Coulter Act Diff
lab B, R	71.7	66.3	Sysmex XN20
lab J	(84.9)	(89.9)	Coulter Act Diff
lab O	(82.8)	(80.3)	CellDyn Emerald
lab P	69.2	67.3	Coulter Act Diff
lab U	74.0	65.6	DellDyn Sapphire
24hr bloods (average)	71.9 x10^6	66.7 x10^6	

Note: mean whole blood PBMC was based on data from 4 labs; data in brackets were excluded, being higher than the cluster data from 4 labs.

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. Small cell clumps present in the thawed vial or after initial centrifugation in specimens from lab B, were possibly neutrophils present after fractionation. After mixing to free cells from these clumps, clumps were removed, and viability observed by haemocytometer was uniformly high (Table 2).

Thawed cell recovery from most specimens was within the required 75-125% range (Figure 1). Post-thaw PBMC recovery was very low in lab B, which was confirmed upon repeat thawing. Low post-thaw recovery from lab B was not associated with an unusually high fractionation recovery, and therefore absolute recovery was also low, also observed with their local donor, and was the reason for failure in this QA round. In lab V, apparent low fractionation recovery with post-thaw recovery >200% suggested counts were out by at least a factor of 2, perhaps a dilution error. Although absolute recovery from lab V was normal, this lab also failed this QA round. In lab J,

fractionation recovery was high, while thawed recovery was marginal, likely the result of high cell counts. When the thawed PBMC count was adjusted up to account for 200-300µl of medium with the cell pellet when resuspended in another 5ml, lab J had thawed recovery within range.

Mean absolute recovery of all PBMC specimens from this round was 48%, suggesting overall proficiency in extraction and cryopreservation of viable PBMC from whole blood samples. 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 is consistent over time. These combined results suggest that the recovery performance standard is appropriate, as the majority of data confirms to expectation.

Functional analysis

PBMC 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). PBMC from both the HIV-pos and HIV-neg IVRN donors gave a weak/undetectable response to the CEF peptide pool, while 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 F and lab K had a high frequency of responder cells in control wells. Since the natural level of background activation in local donor specimens is not known, ELISPOT results from Lab F and K local donor were accepted as a pass, although also being high.

Certification status of participating laboratories after the 35th QA round

All labs achieved uniformly high viability results, and 11 of 12 had good post thaw recovery. Failure of two labs in this QAP round was attributed to incorrect cell counts in lab V, and poor overall recovery of PBMC in lab B. Certification was retained by lab B because of recent good performance. Results from this QA round demonstrate a highly capable network of laboratories certified for participation in clinical studies involving PBMC cryopreservation (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.

35th IVRN QAP report was produced by Dr Wayne Dyer, on behalf of the IVRN Executive.

Table 2. 35th 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
	HIV-pos	8/12/20	30	10	4	40	71.85	55.7	2.000	20.0	11.1	95	6	0	>5000	yes	no	yes	
B	HIV neg	8/12/20	30	10	3	30	66.73	45.0	2.000	20.0	9.0	>95	1	0	>5000	yes	no	yes	fail
	local donor	9/12/20	9	10	1	10	12.42	80.5	5.050	50.5	40.7	>95	10	2590	>5000	high	low	yes	
	HIV-pos	8/12/20	30	8.9	4	35.6	71.85	49.5	8.194	92.1	45.6	>95	4	0	>5000	yes	yes	yes	
E	HIV neg	8/12/20	30	8.3	3	24.9	66.73	37.3	8.874	106.9	39.9	>95	1	0	>5000	yes	yes	yes	pass
	local donor	9/12/20	27	8.7	3	26.1	86.4	30.2	10.206	117.3	35.4	>95	3	30	>5000	yes	yes	yes	
	HIV-pos	8/12/20	30	10.25	4	41	71.85	57.1	10.065	98.2	56.0	>95	75	0	>5000	yes	yes	high control	
F	HIV neg	8/12/20	30	10.75	4	43	66.73	64.4	9.253	86.1	55.5	>95	7	20	>5000	yes	yes	yes	pass
	local donor	9/12/20	27	8.3	3	24.9	64.8	38.4	8.262	99.5	38.3	>95	45	0	>5000	yes	yes	high control	
	HIV-pos	8/12/20	30	10	4.51	45.1	71.85	62.8	7.208	72.1	45.2	>95	7	0	>5000	yes	yes	yes	
J	HIV neg	8/12/20	30	10	4.7	47	66.73	70.4	7.373	73.7	51.9	>95	1	10	>5000	yes	yes	yes	pass
	local donor	9/12/20	20	10	1.7	17	50.7	33.5	7.380	73.8	24.7	>95	1	260	>5000	yes	yes	yes	
	HIV-pos	8/12/20	30	8	5	40	71.85	55.7	8.793	109.9	61.2	>95	83	0	>5000	yes	yes	high control	
K	HIV neg	8/12/20	30	8.6	3	25.8	66.73	38.7	8.390	97.6	37.7	>95	5	0	>5000	yes	yes	yes	pass
	local donor	9/12/20	27	8.5	3	25.5	64.8	39.4	10.269	120.8	47.5	>95	25	0	>5000	yes	yes	high control	
	HIV-pos	8/12/20	30	5.63	8	45.04	71.85	62.7	6.318	112.2	70.3	>95	3	0	>5000	yes	yes	yes	
O	HIV neg	8/12/20	30	5.63	8	45.04	66.73	67.5	6.182	109.8	74.1	>95	0	0	>5000	yes	yes	yes	pass
	local donor	9/12/20	15	5.33	6	31.98	45	71.1	4.850	91.0	64.7	>95	1	1710	>5000	yes	yes	yes	
	HIV-pos	8/12/20	30	8.14	5	40.7	71.85	56.6	8.865	108.9	61.7	>95	4	10	>5000	yes	yes	yes	
P	HIV neg	8/12/20	30	8.58	5	42.9	66.73	64.3	9.253	107.8	69.3	>95	0	10	>5000	yes	yes	yes	pass
	local donor	9/12/20	30	7.05	4	28.2	43.4	65.0	6.860	97.3	63.2	>95	2	720	>5000	yes	yes	yes	
	HIV-pos	8/12/20	30	6.5	5	32.5	71.85	45.2	5.718	88.0	39.8	>95	12	0	>5000	yes	yes	yes	
R	HIV neg	8/12/20	30	6.5	5	32.5	66.73	48.7	6.344	97.6	47.5	>95	1	0	>5000	yes	yes	yes	pass
	local donor	9/12/20	17	5	2	10	23.5	42.6	4.500	90.0	38.3	>95	2	1950	>5000	yes	yes	yes	
	HIV-pos	8/12/20	30	6.03	5	30.15	71.85	42.0	6.149	102.0	42.8	>95	5	20	>5000	yes	yes	yes	
T	HIV neg	8/12/20	30	8.99	5	44.95	66.73	67.4	7.223	80.3	54.1	>95	1	0	>5000	yes	yes	yes	pass
	local donor	9/12/20	22.5	9.4	5	47	NA	OK	6.472	68.9	NA	>95	3	50	>5000	yes	no	yes	
	HIV-pos	8/12/20	30	7.71	4	30.84	71.85	42.9	9.690	125.7	53.9	>95	6	0	>5000	yes	yes	yes	
U	HIV neg	8/12/20	30	8.57	4	34.28	66.73	51.4	9.358	109.2	56.1	>95	0	0	>5000	yes	yes	yes	pass
	local donor	9/12/20	30	9.54	4	38.16	77.8	49.0	11.352	119.0	58.4	>95	2	0	>5000	yes	yes	yes	
	HIV-pos	8/12/20	30	5	2.5	12.5	71.85	17.4	10.956	219.1	38.1	>95	3	10	>5000	low	high	yes	
V	HIV neg	8/12/20	30	5	2.5	12.5	66.73	18.7	12.060	241.2	45.2	>95	0	10	>5000	low	high	yes	fail
	local donor	9/12/20	9	5	1.5	7.5	NA	low	5.357	107.1	NA	>95	1	1060	>5000	low	high	yes	
	HIV-pos	8/12/20	30	7.78	4	31.12	71.85	43.3	7.696	98.9	42.8	>95	9	10	>5000	yes	yes	yes	
W	HIV neg	8/12/20	30	9.92	5	49.6	66.73	74.3	6.839	68.9	51.2	>95	1	0	>5000	high	low	yes	pass
	local donor	9/12/20	18	5.95	2	11.9	55.39	low	7.464	125.4	NA	>95	1	3480	>5000	low	high	yes	

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 results: PMA/Ionomycin: >5000/10^6 PBMC; CEF (mean - 2SD) = 0/10^6 PBMC; control spots (mean +2SD) =12 & 6 spots/well (HIV+ & neg; high outliers excluded).

(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. Post thaw recovery was >75% when 300ul carry-over fluid in tube was included in total count volume.

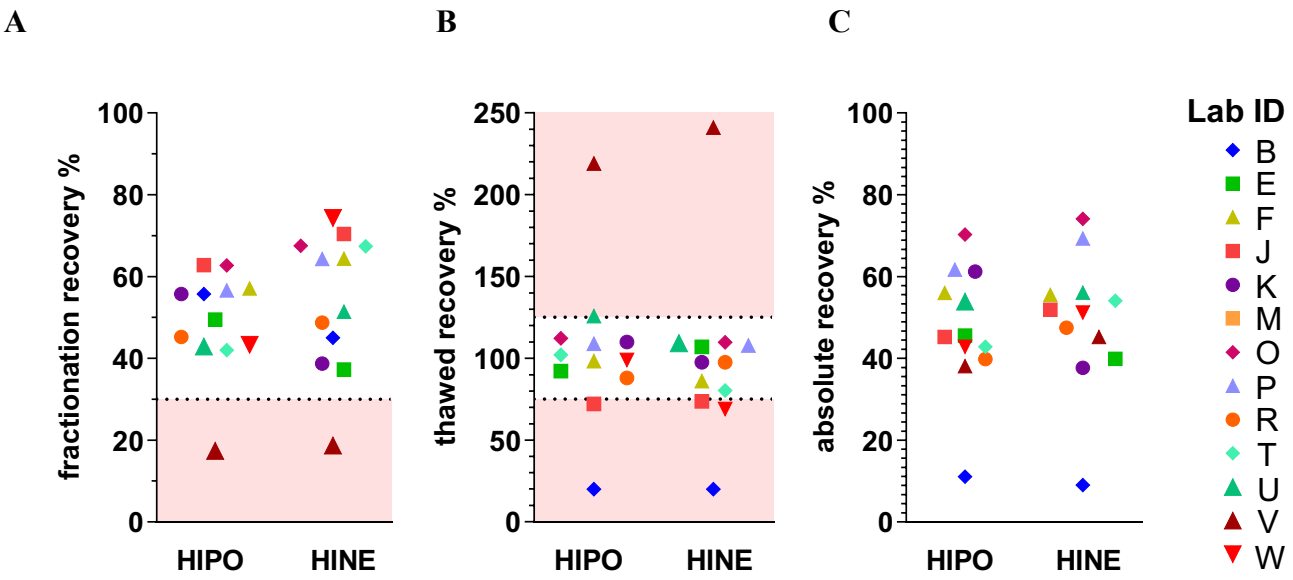

Figure 1. Comparison between relative vs. absolute recovery of PBMC showing (A) post fractionation recovery relative to laboratory cell count; (B) thawed PBMC recovery relative to laboratory cell count, and (C) absolute recovery of PBMC (total thawed PBMC x number of vials) expressed as the % of the mean whole blood PBMC count. Shaded areas in panels A and B define data outside the QA specifications.

Figure 2. 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.

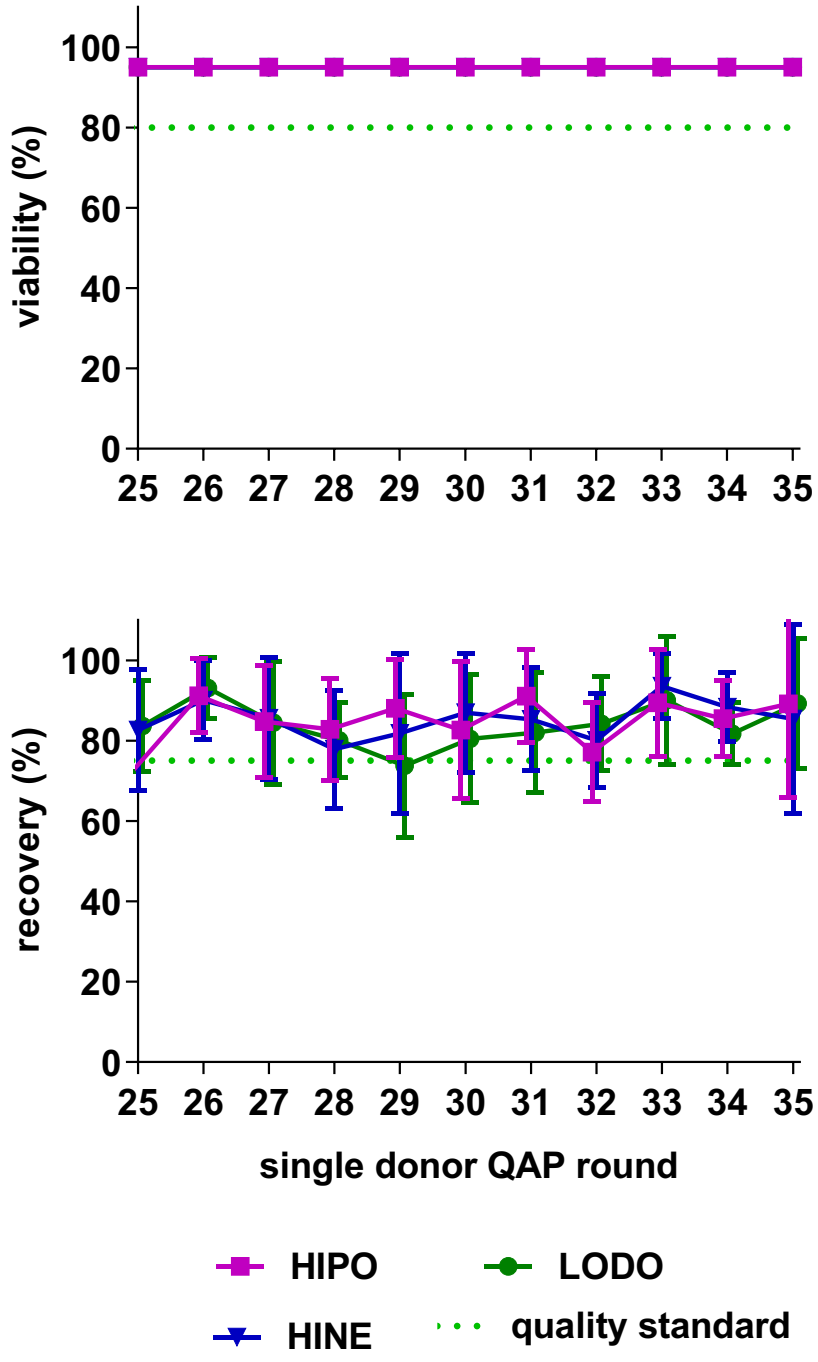

Figure 3. PBMC function results determined by IFN-γ ELISPOT. Antigen-specific responses were determined by stimulation and overnight culture with the CEF peptide pool, and maximal cytokine release with PMA + ionomycin.

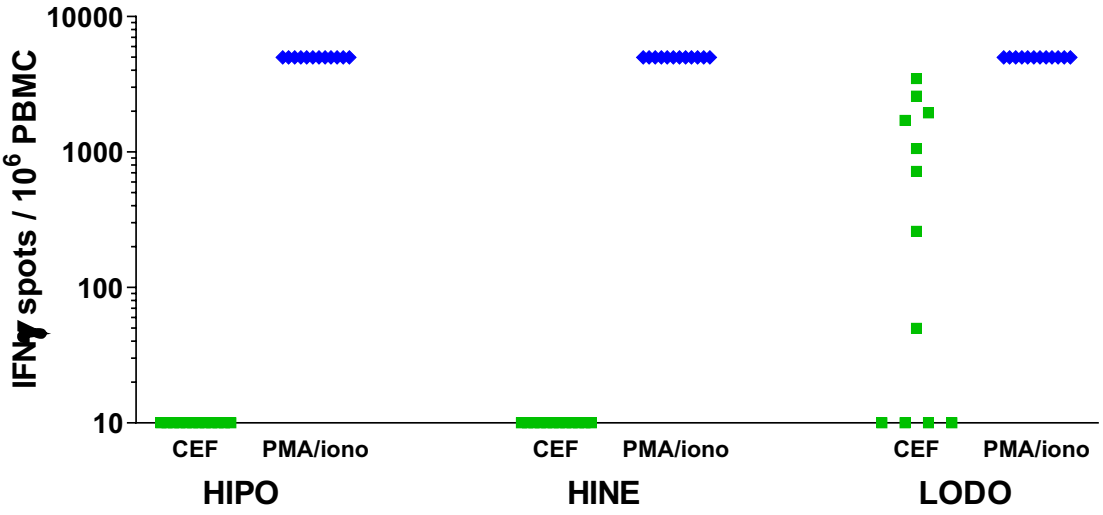

Table 3. Current certification status of Tier 1 labs.

lab code	Adequately performance over the previous QAP rounds? (all 4 quality standards met in at least one PBMC specimen)			current status
	33rd round	34th round	35th round	(passed 2 of 3 QAP rounds)
B	pass	pass	fail	**Certified**
E	pass	pass	pass	**Certified**
F	pass	pass	pass	**Certified**
J	fail	pass	pass	**Certified**
K	pass	pass	pass	**Certified**
M	NA	pass	NA	**Certified**
O	pass	pass	pass	**Certified**
P	pass	pass	pass	**Certified**
R	pass	pass	pass	**Certified**
T	NA	pass	pass	**Certified**
U	pass	pass	pass	**Certified**
V			fail	**not yet certified**
W			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.