Report: 33rd IVRN PBMC cryopreservation QA round, May 2019

The 33rd IVRN QA exercise took place on 21st May 2019, and assessment of returned PBMC specimens took place early June 2019. The primary outcomes of this QA round are:

- Efficient PBMC fractionation;
- Excellent/improved post thaw recovery;
- Acceptable PBMC function despite high background (HIPO) and low response from both IVRN donors;
- 8 out of 9 participating laboratories passed this round.

PBMC fractionation recovery

The number of PBMC in the IVRN blood samples was calculated from full blood differential counts provided from participating labs (Table 1), and used to calculate fractionation recovery (Table 2). The mean fractionation recovery was 58%, which is considered highly efficient. Fractionation recovery from local donor blood was calculated from FBC reported by each lab, or the minimum required recovery was >1 x 10^6 PBMC per 1ml blood if a FBC was not performed.

Table 1. Total PBMC in 30ml whole blood samples for 33rd QA round

Laboratory	HIPO (x10^6/30ml)	HINE (x10^6/30ml)	cell counter
lab B, R	51.63	83.46	Sysmex XN20
lab J	50.19	93.81	Coulter Act Diff
lab K	45.54	72.45	Coulter LH500
lab O	51.03	87.27	CellDyn Emerald
lab P	42.90	90.66	Coulter Act Diff
lab U	44.04	72.78	Cell Dyn Sapphire
mean	47.55 x10^6	83.31 x10^6	

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. Thawed PBMC specimens were free of any cell clumps or debris, and the resulting viability was uniformly high (Table 2).

Post thaw PBMC recovery (thawed PBMC count divided by PBMC in the ampoule) was greatly improved in this QA round. Recovery in all but 3 PBMC specimens was between 75-125%. One exception was Lab E's HINE specimen, which appeared to be a result of underestimation during manual counting, demonstrated by an inverse association between apparently low fractionation recovery and excess post-thaw recovery (Table 2 and Figure 1). Two samples from Lab J with low post thaw recovery had an average fractionation recovery, and therefore the absolute recovery was low (Fig 1C). With the exception of these samples, the absolute recovery of thawed PBMC, expressed as a percentage of total PBMC in fresh blood samples was >40%, suggesting overall proficiency in provision of viable PBMC from blood samples supplied by the IVRN.

The cumulative trend in viability and post-thaw recovery over the past 10 QAP rounds is shown in Figure 2, demonstrating increased post thaw recovery in this QA round.

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). The same IVRN donors was used in this QA round as in the previous QA round, hence responses to the CEF peptide pool were low again. Responses from individual local donors varied from undetectable to strong, as expected. All PBMC samples showed maximal stimulation in the presence of PMA and ionomycin (in excess of 5000 spots/million PBMC). A high frequency of responder cells in control wells may be caused by a level of immune activation in the donor, or exposure to some stimulus during PBMC preparation. High control activation was observed in all HIPO PBMC samples, suggesting this was donor-associated. The same conclusion could be made for the local donor sample from Lab F. However, since nearly all HINE PBMC had low control counts, high background in the HINE sample from Lab J was likely inappropriate cell activation or some other artefact, and therefore this specimen failed the function specifications. All specimens showed uniformly strong response to maximal stimulation with PMA/ionomycin.

Overall conclusions on performance in the 33rd QA round

All labs achieved uniformly high viability results, and overall post thaw recovery was improved over previous QA rounds. Lab J provided 2 of 3 PBMC samples with low post thaw recovery, but the third specimen with acceptable recovery failed the function specification because of high background counts, and therefore all specimens from Lab J failed at least one performance specification. Overall results from this QA round demonstrate a highly capable network of laboratories certified for participation in clinical studies involving PBMC cryopreservation (Table 3). Laboratories that failed more than one QA round out of the past three are classified as being Certified-Under Review, and have the opportunity to participate in remedial action, which may include submission of another PBMC specimen for assessment of desired.

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.

33rd IVRN QAP report was produced by Dr Wayne Dyer, on behalf of the IVRN Executive.

Table 2. 33rd IVRN Single Donor QA Round: PBMC Fractionation Recovery, Viability, Viable Recovery and Function.

			IVRN Tier 1 lab data					QAP coordinator data				PBMC function (ELISPOT)						
lab code	donor category	sample date	blood vol	cells/vial (million)	No. vials	total recovered	[1] fractionation recovery (%	thawed cell count (X10^6)	[3] post thaw recovery (%)	[6] absolute recovery (%	[2] viability %	**control** spots/wel	**net spots/10^6 PBMC** CEF	PMA/Iono	[1] Adequate PBMC fractionated	Adequate viability/recovery	[4] Adequate response in function assays	[5] overall result
B	HIV-pos	20/5/19	30	9.8	2	19.6	41.2	9.680	98.8	40.7	>95	22	0	>5000	yes	yes	yes	
	HIV neg	20/5/19	30	8.83	4	35.32	42.4	10.217	115.7	49.1	>95	4	40	>5000	yes	yes	yes	pass
	local donor	21/5/19	30	7.75	2	15.5	46.1	7.840	101.2	46.7	>95	2	0	>5000	yes	yes	yes	
E	HIV-pos	20/5/19	30	9.75	2	19.5	41.0	11.436	117.3	48.1	>95	4	60	>5000	yes	yes	yes	
	HIV neg	20/5/19	30	9.25	2	18.5	22.2	12.545	135.6	30.1	>95	1	40	>5000	no	yes	yes	pass
	local donor	21/5/19	27	7.25	2	14.5	low	6.755	93.2	NA	>95	5	570	>5000	yes	yes	yes	
F	HIV-pos	20/5/19	30	10	3	30	63.1	7.672	76.7	48.4	>95	100	120	>5000	yes	yes	yes	
	HIV neg	20/5/19	30	10	5	50	60.0	10.364	103.6	62.2	>95	16	0	>5000	yes	yes	yes	pass
	local donor	21/5/19	30	10	4	40	OK	9.710	97.1	NA	92	48	0	>5000	yes	yes	yes	
J	HIV-pos	20/5/19	30	9	2	18	37.9	5.514	61.3	23.2	>95	111	0	>5000	yes	no	yes	
	HIV neg	20/5/19	30	10	4	40	48.0	7.776	77.8	37.3	>95	39	0	>5000	yes	yes	no	pass
	local donor	21/5/19	20	8	2	16	50.0	4.000	50.0	25.0	>95	6	340	>5000	yes	no	yes	
K	HIV-pos	20/5/19	30	7	4	28	58.9	6.377	91.1	53.6	>95	17	10	>5000	yes	yes	yes	
	HIV neg	20/5/19	30	8.3	6	49.8	59.8	8.309	100.1	59.8	>95	0	10	>5000	yes	yes	yes	pass
	local donor	21/5/19	27	7.75	4	31	67.8	6.902	89.1	60.4	>95	8	660	>5000	yes	yes	yes	
M																		
O	HIV-pos	20/5/19	30	6	5	30	63.1	6.615	110.3	69.6	>95	32	0	>5000	yes	yes	yes	
	HIV neg	20/5/19	30	8.3	7	58.1	69.7	6.860	82.7	57.6	>95	3	10	>5000	yes	yes	yes	pass
	local donor	21/5/19	15.5	6.33	6	37.98	93.3	5.319	84.0	78.4	>95	6	2430	>5000	yes	yes	yes	
P	HIV-pos	20/5/19	30	9	3	27	56.8	9.840	109.3	62.1	>95	14	50	>5000	yes	yes	yes	
	HIV neg	20/5/19	30	9.3	7	65.1	78.1	8.883	95.5	74.6	>95	3	0	>5000	yes	yes	yes	pass
	local donor	21/5/19	31	8.3	3	24.9	60.0	9.301	112.1	67.3	>95	5	1090	>5000	yes	yes	yes	
R	HIV-pos	20/5/19	30	6.46	5	32.3	67.9	5.337	82.6	56.1	>95	18	0	>5000	yes	yes	yes	
	HIV neg	20/5/19	30	6.41	10	64.1	76.9	5.850	91.3	70.2	>95	2	30	>5000	yes	yes	yes	pass
	local donor	21/5/19	30	6.55	2	13.1	39.0	6.390	97.6	38.0	>95	3	0	>5000	yes	yes	yes	
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U	HIV-pos	20/5/19	30	10	3	30	63.1	9.390	93.9	59.2	>95	32	80	>5000	yes	yes	yes	
	HIV neg	20/5/19	30	9.49	6	56.94	68.3	8.930	94.1	64.3	>95	6	0	>5000	yes	yes	yes	pass
	local donor	21/5/19	31.4	8.98	8	71.84	71.9	9.035	100.6	72.4	>95	8	2930	>5000	yes	yes	yes	

Notes: **(1) Assessment criteria 1:** The minimum required fractionation recovery was 30% of available PBMC, which averaged 47.55 million PBMC/30ml blood from the HIV-pos and 83.31 million from HIV-neg donor. Local donor fractionation efficiency was based on whole blood counts provided by each lab, or at least 1x10^6 PBMC/ml blood if whole blood counts were 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. Cell counts performed on a Coulter Act Diff haematology cell counter.

(4) Assessment criteria 4: ELISPOT results: PMA/Ionomycin: >5000/10^6 PBMC (all samples); CEF (mean - 2SD) = 0/10^6 PBMC (HIV+ & neg); control spots (mean +2SD) <117 & <32 spots/well (HIV+ & neg).

(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 shading indicate results that are outside the performance standards.

Orange shading shows the outcome of an underestimated PBMC count, resulting in an apparent low fractionation recovery and too many cells dispensed in each ampoule.

Background IFN-gamma spots were high in HIPO samples.

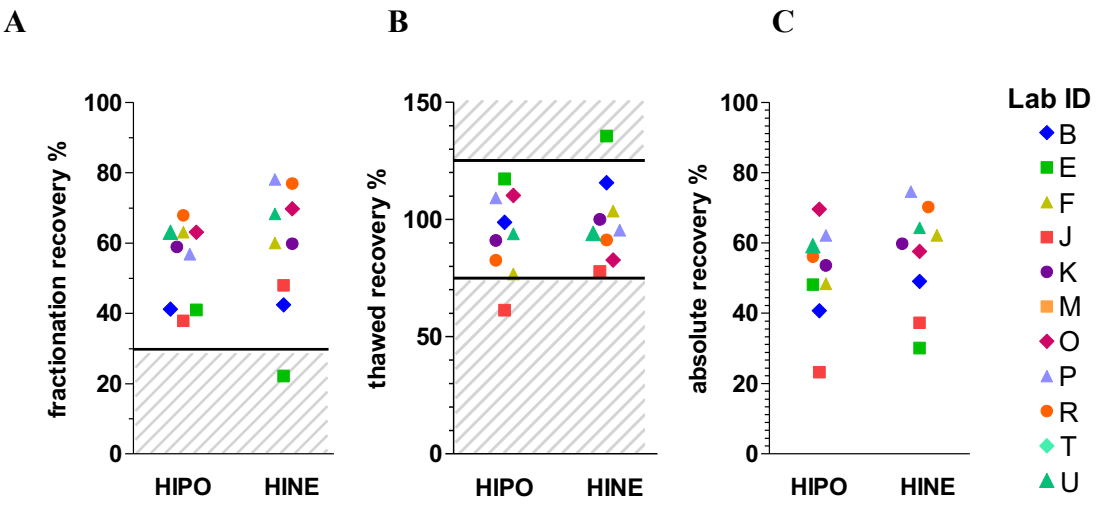

Figure 1. Comparison of relative vs. absolute recovery of PBMC showing (A) post fractionation recovery relative to laboratory cell count; (B) thawed PBMC recovery relative to laboratory cell count, and (C) absolute recovery of PBMC (total thawed PBMC x number of vials) expressed as the % of the mean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

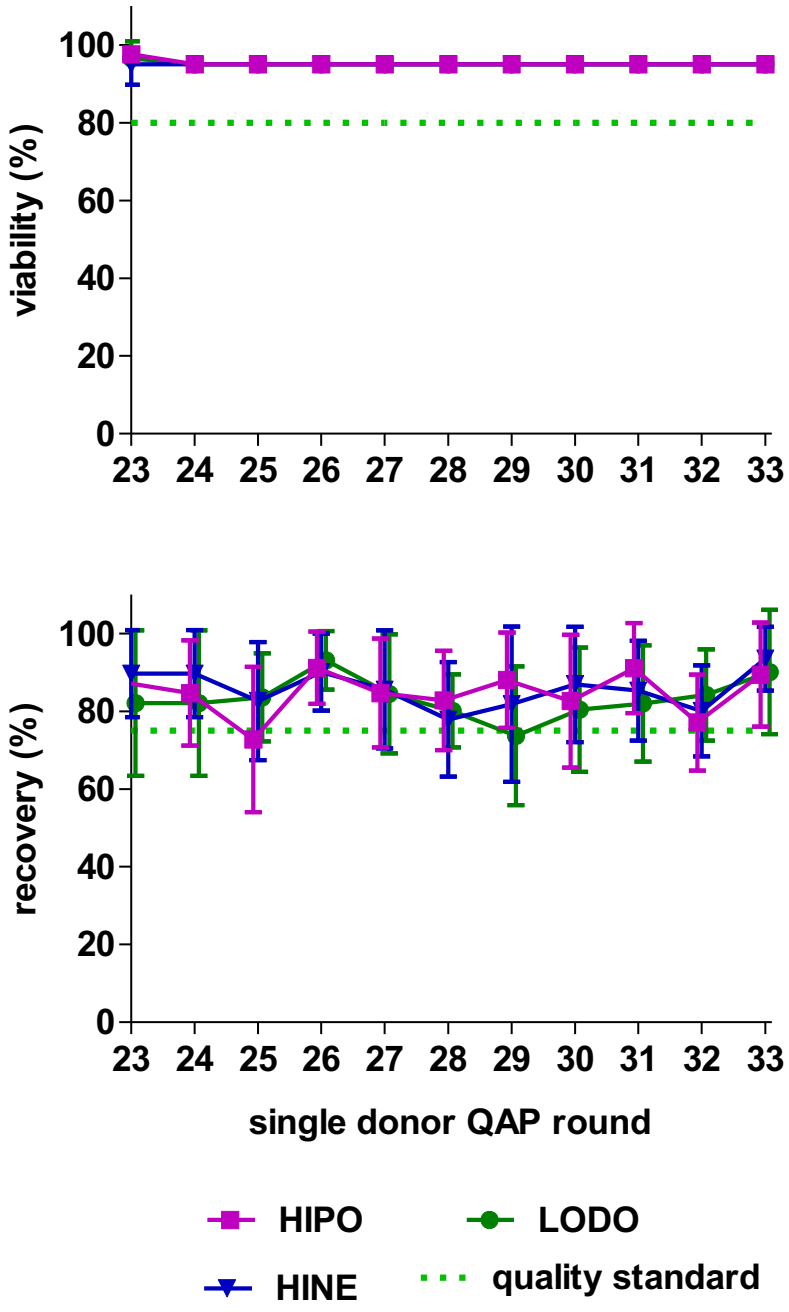

Figure 2. Cumulative trend in viability and post thaw recovery compared with the 10 previous QA rounds.
Mean and standard deviation; post thaw recovery results >100% were reported as 100%.

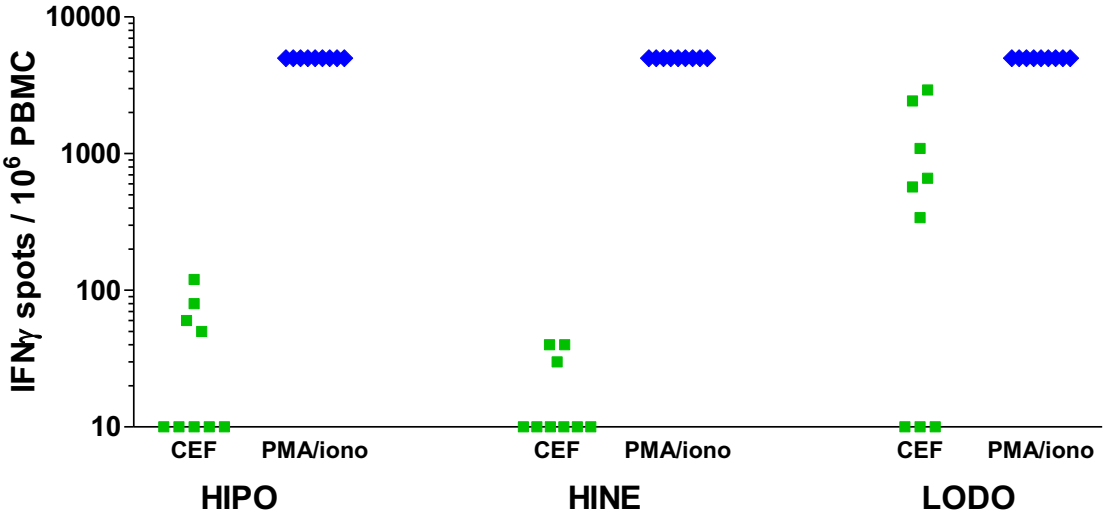

Figure 3. PBMC function results determined by IFN-γ ELISPOT. Antigen-specific responses were determined by stimulation and overnight culture with the CEF peptide pool, and maximal cytokine release with PMA + ionomycin.

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
	31st round	32nd round	33rd round	(passed 2 of 3 QAP rounds)
B	pass	pass	pass	**Certified**
E	pass	pass	pass	**Certified**
F	fail	pass	pass	**Certified**
J	pass	pass	fail	**Certified**
K	pass	fail	pass	**Certified**
M	NA	fail	NA	**Certified – Under Review**
O	pass	pass	pass	**Certified**
P	pass	pass	pass	**Certified**
R	pass	pass	pass	**Certified**
T		pass	NA	**Certified**
U			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.