Report on the 28th IVRN PBMC cryopreservation QA round, November 2016

Blood was taken from the IVRN donors on 14th November 2016 for processing the following morning along with a freshly obtained local blood sample at each laboratory. Crypreserved PBMC specimens were returned the same week, and assessments conducted on 20th and 21st November.

PBMC fractionation recovery

Laboratories with access to an automated counter that could quantify both lymphocytes + monocytes provided full blood counts which were averaged for calculating total PBMC in the 30ml blood samples (Table 1). The whole blood counts provided by Lab O were considered outliers and hence excluded from the average (Table 1). All laboratories achieved at least 30% fractionation efficiency from at least one blood specimen (Table 2). The mean fractionation efficiency for all specimens processed was 58%, suggesting highly efficient recovery of PBMC.

Table 1. Total PBMC in 30ml donor blood samples for 28th QA round.

Laboratory	HIPO ($x10^6$/30ml)	HINE ($x10^6$/30ml)	cell counter	
lab B, R	69.83	81.96	CellDyn Sapphire	
lab J	63.68	-	Coulter Act Diff	
lab K	58.96	72.33	Coulter Max M	
lab M	64.26	74.76	Sysmex XE5000	
lab O	38.62	18	CellDyn Emerald	excluded from average
Lab P	62.46	75.92	Coulter Act Diff	
fresh blood	63.68	74.89	Coulter Act Diff	
mean	**63.84 $x10^6$/30ml**	**76.24 $x10^6$/ml**		
30% recovery	**19.15 $x10^6$**	**22.87 $x10^6$**		

Counting accuracy

Post-thaw recovery was variable, and as in previous QA rounds, low recovery was associated with overestimation of the number of fractionated PBMC before cryopreservation. The stand-out example from this QA round was the HIV-neg specimen from Lab F. More PBMC were counted than what were present in the whole blood sample. This overestimation of fractionated PBMC resulted in much less cells in each ampoule than expected and hence the low post-thaw recovery. These out-of-range results when combined gave an excellent absolute recovery of 61.3% from this specimen (Table 1 and Figure 1). However, the requirement for producing cryopreserved PBMC within a tight band of numerical accuracy is needed for maximising return from precious clinical specimens, and therefore remains the basis for acceptable performance in this QAP.

PBMC viability and recovery

The viability of all thawed PBMC specimens was >95% (Table 2), as determined by visual inspection of cells in the presence of trypan blue, and confirmed by manual counting of selected specimens. All participating IVRN laboratories have provided consistent high viability PBMC for many years. The cumulative trend in post-thaw viability and recovery over the past 10 QAP rounds is shown in Figure 2. Although there was a measurable decrease in the average recovery from previous QA rounds, the overall proficiency of the IVRN Tier 1 Laboratory Network in processing PBMC from day-old transported whole blood specimens is within specification.

Functional analysis

The IFNγ ELISPOT assay was used to determine PBMC function, in response to antigenic stimulation with the CEF peptide pool (representative peptide epitopes from CMV, EBV and Influenza), and maximal stimulation from PMA and ionomycin (Figure 3). As in previous QA rounds, PBMC from the HIV+ donor did not respond to the CEF peptide pool, whereas responses from the HIV-negative donor PBMC were consistent. As usual, a wider variation in responses from individual local donors was noted. All PBMC samples showed maximal stimulation in the presence of PMA and ionomycin (in excess of 5000 spots/million PBMC). However, the background spots in control wells were very high for some specimens (data shaded orange, Table 2), on average higher than observed in previous QA rounds. Spontaneous IFN-γ production is normally much lower than 50 spots/well. However, high background did not appear to result in reduced responses to CEF peptides for IVRN specimens. The prevalence of high background responses meant that it was not appropriate to use background counts as part of the proficiency assessment in this QA round, given the lack of significant effect on CEF responses.

Overall conclusions on performance in the 28th QA round

All labs achieved uniformly high viability results, whereas recovery of PBMC is variable between labs, and is dependent on accuracy of cell counting and procedures to minimise cell loos during centrifuging. The absolute recovery of PBMC suggests that all labs can fractionate and cryopreserve sufficient quality PBMC from the available blood samples. The IVRN Tier 1 Lab network can therefore claim to have the highest of international standards for PBMC fractionation and cryopreservation, with highly capable laboratories around the country certified for participation in clinical studies involving PBMC cryopreservation (Table 3).

Thanks for your ongoing participation in the IVRN PBMC processing QAP, and contributing to this national network of clinical trial support labs. 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 staff join your group, time should be set aside for specimen processing scientists to self assess their performance between QA rounds. All are encouraged to discuss any methods or performance issues with the QAP coordinator.

28th IVRN QAP report was produced by Dr Wayne Dyer, on behalf of the IVRN Executive.

Table 1. 28th 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/well	**net spots/10^6 PBMC** CEF	PMA/Iono	[1] Adequate PBMC fractionated	Adequate viability/recovery	[4] Adequate response in function assays	[5] overall result
	HIV-pos	14/11/16	30	9.1	4	36.4	57.0	5.407	59.4	33.9	>95	46	0	>5000	yes	no	yes	
B	HIV neg	14/11/16	30	9.36	5	46.8	61.4	5.467	58.4	35.8	>95	23	847	>5000	yes	no	yes	fail
	local donor	15/11/16	18	8.05	2	16.1	24.6	5.868	72.9	17.9	>95	140	1720	>5000	yes	no	yes	
	HIV-pos	14/11/16	30	10.125	4	40.5	63.4	7.425	73.3	46.5	>95	26	0	>5000	yes	no	yes	
E	HIV neg	14/11/16	30	10.8	5	54	70.8	8.937	82.8	58.6	>95	15	1180	>5000	yes	yes	yes	pass
	local donor	14/11/16	18	10.25	2	20.5	46.1	8.407	82.0	37.8	>95	370	0	>5000	yes	yes	yes	
	HIV-pos	14/11/16	30	9.9	4	39.6	62.0	8.784	88.7	55.0	>95	109	0	>5000	yes	yes	yes	
F	HIV neg	14/11/16	30	9.8	8	78.4	102.8	5.844	59.6	61.3	>95	61	830	>5000	yes	no	yes	pass
	local donor	15/11/16	27	9.7	5	48.5	OK	6.455	66.5	NA	>95	68	160	>5000	yes	no	yes	
	HIV-pos	14/11/16	30	5	4	20	31.3	4.000	80.0	25.1	>95	23	20	>5000	yes	yes	yes	
J	HIV neg	14/11/16	30	10	4.26	42.6	55.9	6.468	64.7	36.2	>95	8	770	>5000	yes	no	yes	pass
	local donor	15/11/16	24	10	2.5	25	56.2	7.896	79.0	44.4	>95	6	590	>5000	yes	yes	yes	
	HIV-pos	14/11/16	30	7.1	6	42.6	66.7	5.916	83.3	55.6	89	21	10	>5000	yes	yes	yes	
K	HIV neg	14/11/16	30	8.1	7	56.7	74.4	5.970	73.7	54.8	>95	3	820	>5000	yes	no	yes	pass
	local donor	15/11/16	27	7.6	5	38	61.7	6.435	84.7	52.3	>95	10	20	>5000	yes	yes	yes	
	HIV-pos	14/11/16	30	6.09	4	24.36	38.2	6.422	105.5	40.3	>95	14	0	>5000	yes	yes	yes	
M	HIV neg	14/11/16	30	5.03	6	30.18	39.6	4.500	89.5	35.4	>95	30	520	>5000	yes	yes	yes	pass
	local donor	15/11/16	48	11.41	6	68.46	69.6	8.347	73.2	50.9	>95	65	0	>5000	yes	no	yes	
	HIV-pos	14/11/16	30	6.3	5	31.5	49.3	4.860	77.1	38.0	>95	22	20	>5000	yes	yes	yes	
O	HIV neg	14/11/16	30	8.5	3	25.5	33.4	6.951	81.8	27.4	>95	14	550	>5000	yes	yes	yes	pass
	local donor	15/11/16	16	9	3	27	74.5	7.425	82.5	61.5	>95	4	10	>5000	yes	yes	yes	
	HIV-pos	14/11/16	30	8	4	32	50.1	8.910	111.3	55.8	>95	21	0	>5000	yes	yes	yes	
P	HIV neg	14/11/16	30	9.2	5	46	60.3	9.910	107.7	65.0	>95	10	1480	>5000	yes	yes	yes	pass
	local donor	15/11/16	22	8.9	4	35.6	77.4	8.973	100.8	78.0	>95	8	20	>5000	yes	yes	yes	
	HIV-pos	14/11/16	30	5.9	6	35.4	55.5	4.945	83.8	46.5	>95	12	0	>5000	yes	yes	yes	
R	HIV neg	14/11/16	30	6.6	7	46.2	60.6	5.982	90.6	54.9	>95	4	1020	>5000	yes	yes	yes	pass
	local donor	15/11/16	17.5	5	3	15	39.2	4.000	80.0	31.4	>95	75	2390	>5000	yes	yes	yes	

Notes: **(1) Assessment criteria 1:** The minimum required fractionation recovery was 30% of available PBMC, which averaged 63.84 million PBMC/30ml blood from HIV-pos and 76.24 million/30ml blood 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, counted in a haemacytometer.

(3) Assessment criteria 3: Required recovery of viable cells: >75% and <125% of stated vial contents. Cell counts performed on a Coulter Act Diff cell counter.

(4) Assessment criteria 4: ELISPOT results: PMA/Ionomycin: >5000/10^6 PBMC (all samples); CEF (mean - 2SD) >0 & >200/10^6 PBMC (HIV+ & neg); control (mean +2SD) <93 & <55 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 indicates results outside specification, not applied as a performance standard.

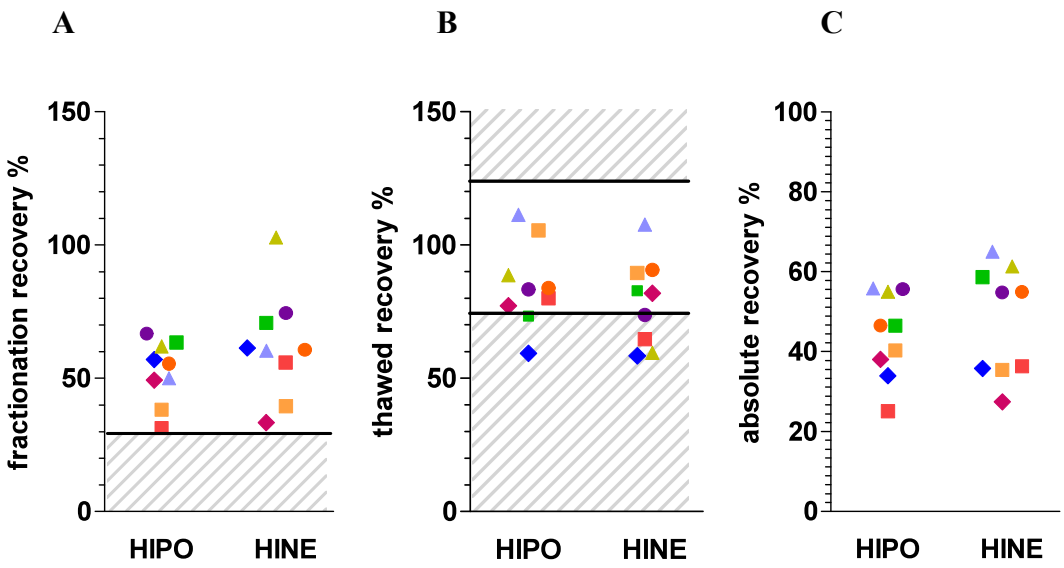

Figure 1. Comparison of relative vs. absolute recovery of PBMC showing (A) post fractionation recovery relative to laboratory cell count; (B) thawed PBMC recovery relative to laboratory cell count, and (C) absolute recovery of PBMC expressed as the % of the mean whole blood PBMC count. Shaded areas in panels A and B define data outside the QA specifications. Data from each laboratory is represented by the same symbol between panels.

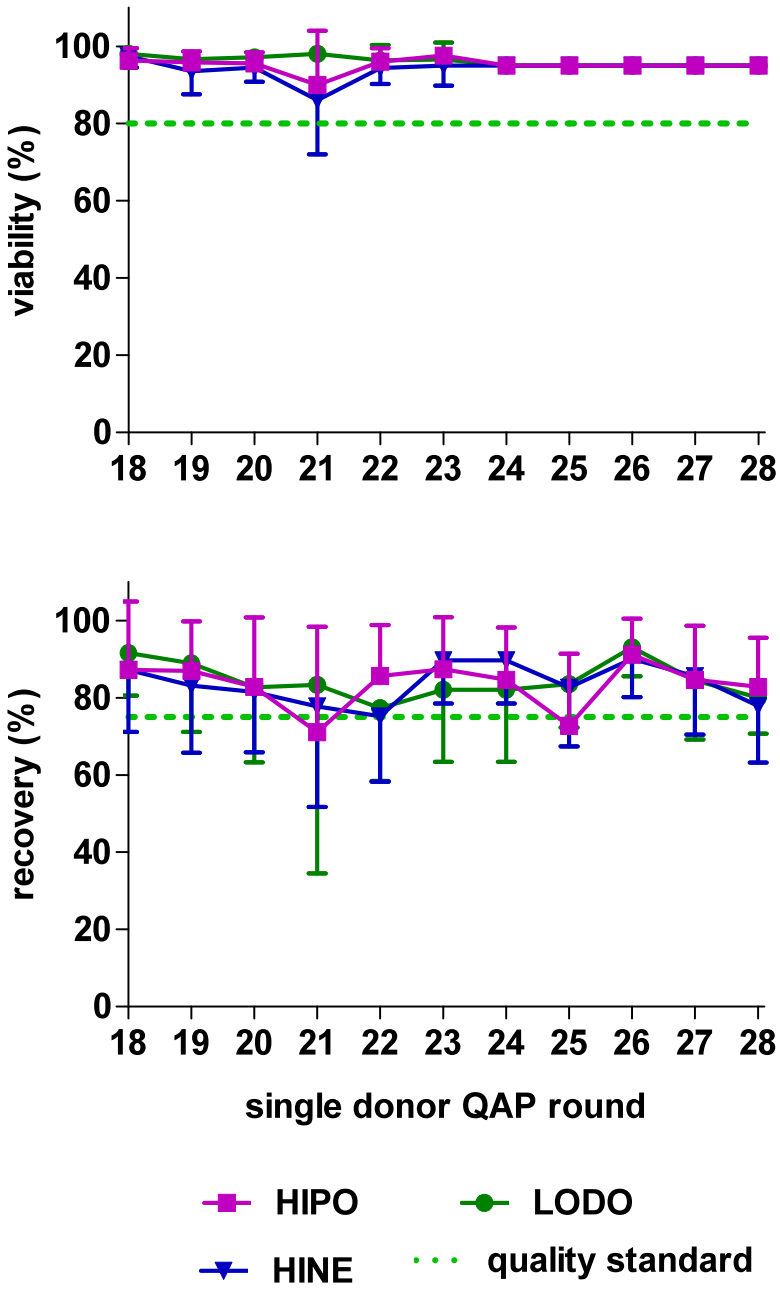

Figure 2. Cumulative trend in viability and post thaw recovery compared with the 10 previous QA rounds.
Mean and standard deviation; recovery results >100% were rounded down to a maximum recovery of 100%.

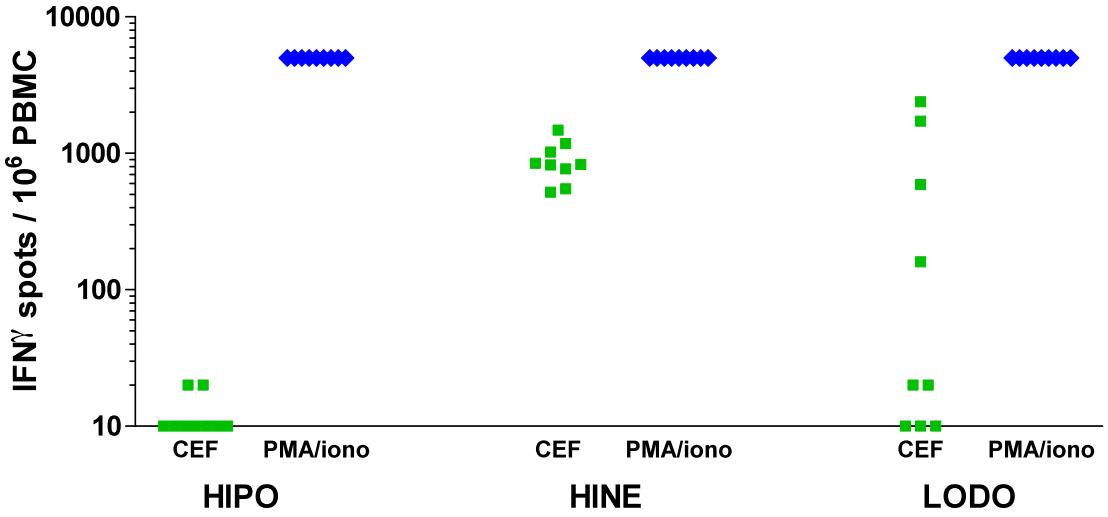

Figure 3. PBMC function results determined by IFN-γ ELISPOT. Antigen-specific responses were determined by stimulation and overnight culture with the CEF peptide pool, and maximal cytokine release with PMA + ionomycin.

Table 3. Current certification status of Tier 1 labs.

lab code	Performed adequately over the previous QAP rounds? (all 4 quality standards met in at least one PBMC specimen)			current status
	26th round	27th round	28th round	(passed 2 of 3 QAP rounds)
B	yes	yes	no	**Certified**
E	yes	yes	yes	**Certified**
F	yes	yes	yes	**Certified**
J	yes	yes	yes	**Certified**
K	yes	yes	yes	**Certified**
M	yes	yes	yes	**Certified**
O	yes	yes	yes	**Certified**
P	yes	yes	yes	**Certified**
R	yes	yes	yes	**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. 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.