Report on the 27th IVRN Single Donor PBMC QA round, April 2016

Blood was taken from the IVRN donors on 17th April 2016 for processing the following morning along with a freshly obtained local blood sample at each laboratory. There are currently 9 participating IVRN Tier 1 laboratories, and all passed the required proficiency measures for this QA round and therefore remain certified for IVRN sponsored PBMC cryopreservation activities.

PBMC fractionation recovery and counting accuracy

Laboratories with access to an automated counter that could quantify both lymphocytes + monocytes provided full blood counts that were used to calculate total PBMC in the 30ml blood sample shipped to each lab for the exercise (Table 1). Based on the mean PBMC content of the IVRN blood specimens, all laboratories achieving at least 30% fractionation efficiency from at least one blood specimen (Table 2). The mean fractionation efficiency for all specimens processed was 55%, the same recorded in the previous QA round, demonstrating highly efficient recovery of PBMC.

Table 1. Total PBMC in 30ml donor blood samples for 27th QA round.

Laboratory	HIPO ($x10^6$/ml)	HINE ($x10^6$/ml)	cell counter
lab B, R	1.769	3.118	CellDyn Sapphire
lab J	1.9	3.5	Coulter Act Diff
lab K	2	2.8	Coulter Max M
lab M	1.73	3.16	Sysmex XE5000
lab O	1.4	2.8	CellDyn Emerald
Lab P	1.8	3.2	Coulter Act Diff
fresh blood	1.769	3.002	Coulter Act Diff
mean	**1.767 $x10^6$/ml**	**3.083 $x10^6$/ml**	
Total/30ml	**53.0 $x10^6$**	**90.2 $x10^6$**	
30% recovery	**15.9 $x10^6$**	**27.75 $x10^6$**	

Cell counting accuracy continues to improve. Laboratories J and P appear to have miscalculated cell number or sample volume in one of their specimens, reporting an apparent reduced fractionation recovery with a correspondingly excessive thaw recovery. Conversely, one of Lab F's PBMC counts was highly overestimated resulting in a low thaw recovery. These out-of-range results when combined gave an acceptable absolute recovery (Table 1 and Figure 2).

Interestingly, the absolute recovery of PBMC was on average lower for the HIV+ donor blood samples, suggesting that leukocyte quality may have been reduced in blood from this donor, possibly contributing to the overall lower fractionation recoveries compared with the HIV-neg blood sample (Table 1). Reduced blood sample quality may also be evident from the lack of response to the CEF peptide pool measured by ELISPOT (Figure 3), because PBMC from this donor had responded to the CEF peptides in previous QA rounds.

PBMC viability and recovery

The viability of nearly all thawed PBMC specimens was >95% (Table 2), as determined by visual inspection of all samples 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. The overall proficiency of the IVRN Tier 1 Laboratory Network in processing PBMC from day-old transported whole blood specimens remains excellent.

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). In this round, PBMC from the HIV+ donor did not respond to the CEF peptide pool. A good response to CEF was obtained from PBMC from this individual when he donated blood for the previous QA round. As usual, a wider variation in responses from individual local donors was noted. However, all PBMC samples showed maximal stimulation in the presence of PMA and ionomycin (in excess of 5000 spots/million PBMC), and all had low background responses in the control wells, as expected from good quality functional PBMC.

Overall conclusions on performance in the 27th QA round

All labs achieved uniformly high viability results, and post thaw recovery results continue to improve, showing a uniformly high standard across all laboratories. 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.

27th IVRN QAP report was produced by Dr Wayne Dyer, on behalf of the IVRN Executive.

Table 1. 27th 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
B	HIV-pos	18/04/16	30	8.1	3	24.3	45.8	8.865	109.4	50.1	>95	12	0	>5000	yes	yes	yes	pass
	HIV neg	18/04/16	30	9.38	6	56.3	60.9	9.415	100.4	61.1	>95	1	1030	>5000	yes	yes	yes	
	local donor	19/04/16		10.1	1	10.1	50.2	5.838	57.8	29.0	>95	1	120	>5000	yes	no	yes	
E	HIV-pos	18/04/16	30	9.42	3	28.26	53.3	6.902	73.3	39.1	>95	2	0	>5000	yes	no	yes	pass
	HIV neg	18/04/16	30	10.4	5	52	56.2	8.910	85.7	48.1	>95	2	1130	>5000	yes	yes	yes	
	local donor	19/04/16		9.25	4	37	44.8	8.441	91.3	40.9	>95	0	680	>5000	yes	yes	yes	
F	HIV-pos	18/04/16	30	9.7	4	38.8	73.2	6.839	70.5	51.6	>95	11	0	>5000	yes	no	yes	pass
	HIV neg	18/04/16	30	12.5	10	125	135.1	6.455	51.6	69.8	>95	6	1020	>5000	high	no	yes	
	local donor	19/04/16		10	7	70	OK	8.901	89.0		>95	5	0	>5000	yes	yes	yes	
J	HIV-pos	18/04/16	30	7.5	2	15	28.3	7.230	96.4	27.3	>95	5	0	>5000	no	yes	yes	pass
	HIV neg	18/04/16	30	9.3	6	55.8	60.3	7.433	79.9	48.2	>95	4	1350	>5000	yes	yes	yes	
	local donor	19/04/16		8	2	16	42.1	9.424	117.8	49.6	>95	4	650	>5000	yes	yes	yes	
K	HIV-pos	18/04/16	30	7.6	3	22.8	43.0	4.820	63.4	27.3	89	8	0	>5000	yes	no	yes	pass
	HIV neg	18/04/16	30	8	6	48	51.9	6.383	79.8	41.4	>95	3	890	>5000	yes	yes	yes	
	local donor	19/04/16		7.28	4	29.1	46.9	4.500	61.8	29.0	>95	15	330	>5000	yes	no	yes	
M	HIV-pos	18/04/16	30	6.6	3	19.8	37.4	5.838	88.5	33.1	>95	5	0	>5000	yes	yes	yes	pass
	HIV neg	18/04/16	30	6.85	6	41.1	44.8	7.425	108.4	48.6	>95	0	650	>5000	yes	yes	yes	
	local donor	19/04/16		6.73	6	40.38	71.0	5.934	88.2	62.6	>95	3	710	>5000	yes	yes	yes	
O	HIV-pos	18/04/16	30	8.25	2	16.5	31.1	7.760	94.1	29.3	>95	3	0	>5000	yes	yes	yes	pass
	HIV neg	18/04/16	30	9.6	5	48	51.9	8.432	87.8	45.6	>95	1	920	>5000	yes	yes	yes	
	local donor	19/04/16		7.5	2	15	55.1	6.930	92.4	50.9	>95	1	810	>5000	yes	yes	yes	
P	HIV-pos	18/04/16	30	7.1	2	14.2	26.8	9.810	138.2	37.0	>95	7	0	>5000	no	high	yes	pass
	HIV neg	18/04/16	30	10	4	40	43.2	14.460	144.6	62.5	>95	1	1140	>5000	yes	high	yes	
	local donor	19/04/16		10	3	30	50.0	10.440	104.4	52.2	>95	3	140	>5000	yes	yes	yes	
R	HIV-pos	18/04/16	30	6.4	5	32	60.4	4.890	76.4	46.1	>95	5	0	>5000	yes	yes	yes	pass
	HIV neg	18/04/16	30	6.4	11	70.4	76.1	5.467	85.4	65.0	>95	3	970	>5000	yes	yes	yes	
	local donor	19/04/16		5	3	15	93.2	4.000	80.0	74.6	>95	6	200	>5000	yes	yes	yes	

Notes: **(1) Assessment criteria 1:** The minimum required fractionation recovery was 30% of available PBMC (see Table 1 for reported full blood counts).
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 & >522/10^6 PBMC (HIV+ & neg); control (mean +2SD) <13.2 & <6.2 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.

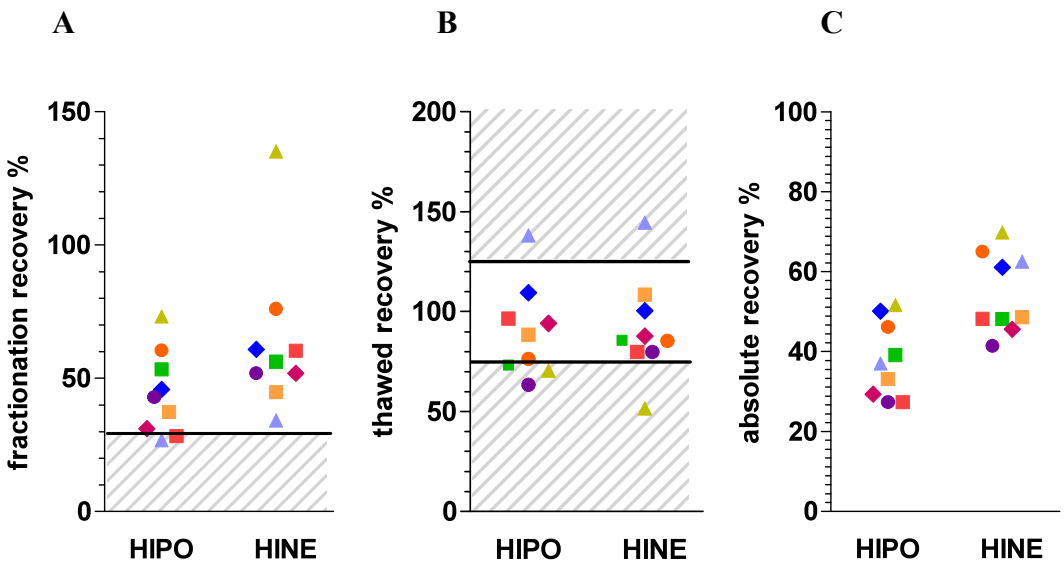

Figure 1. Comparison of relative vs. absolute recovery of PBMC showing (A) post fractionation recovery relative to laboratory cell count; (B) thawed PBMC recovery relative to laboratory cell count, and (C) absolute recovery of PBMC expressed as the % of the mean whole blood PBMC count. Shaded areas in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

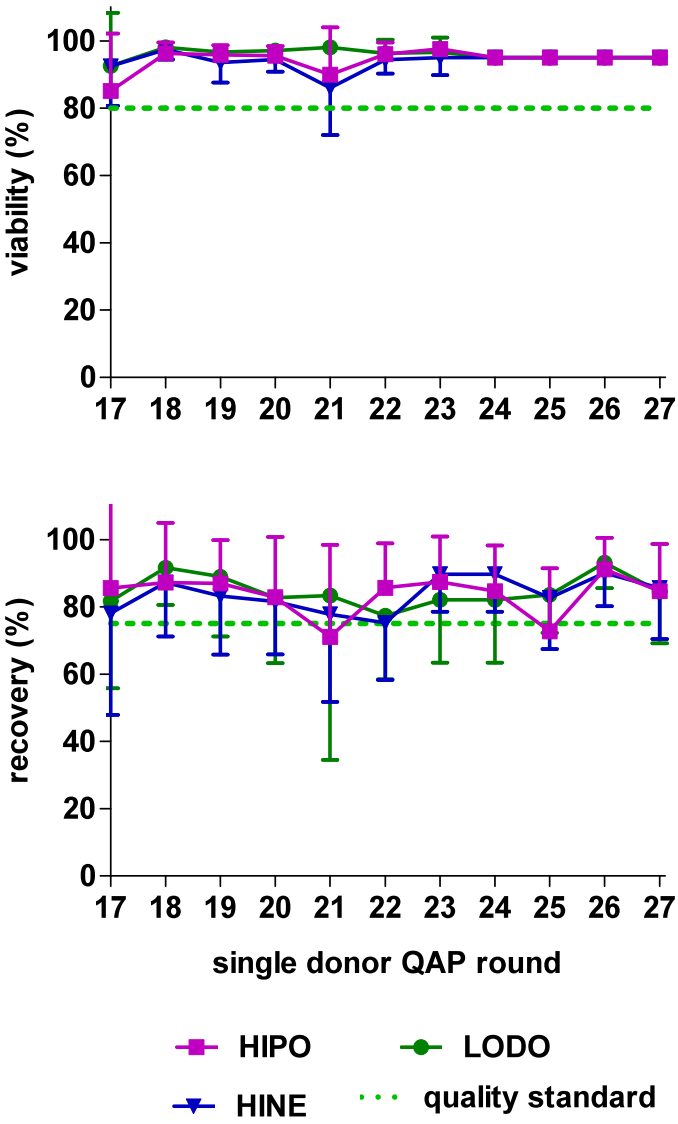

Figure 2. Cumulative trend in viability and post thaw recovery compared with the 10 previous QA rounds.
Mean and standard deviation; recovery results >100% were rounded down to a maximum recovery of 100%.

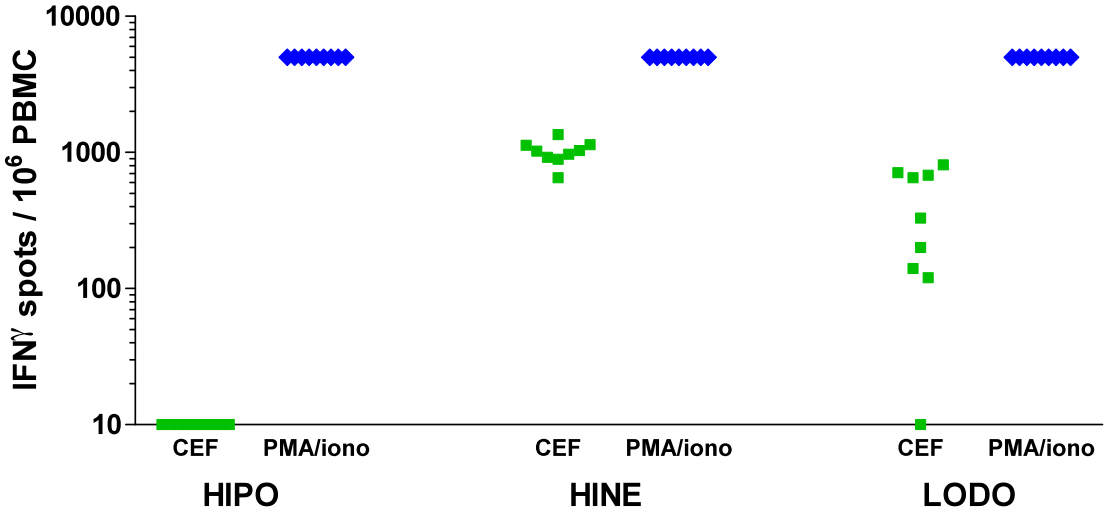

Figure 3. PBMC function results determined by IFN-γ ELISPOT. Antigen-specific responses were determined by stimulation and overnight culture with the CEF peptide pool, and maximal cytokine release with PMA + ionomycin.

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
	25th round	26th round	27th round	(passed 2 of 3 QAP rounds)
B	no	yes	yes	**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	no	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.