Report on the 26th IVRN Single Donor PBMC QA round, Nov 2015

Blood was taken from the IVRN donors on 11th November 2015 for processing the following morning along with a freshly obtained local blood sample at each laboratory. All 10 registered IVRN Tier laboratories participated in this QA round, and all laboratories passed the required proficiency measures and are therefore certified for IVRN sponsored PBMC cryopreservation.

PBMC recovery and counting accuracy

Laboratories with access to an automated counter that could quantify both lymphocytes + monocytes provided full blood counts to calculate total PBMC in each 30ml blood sample provided 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%, demonstrating highly efficient recovery of PBMC.

Table 1. Total PBMC in 30ml donor blood samples for 26th QA round.

Laboratory	HIPO ($x10^6$/ml)	HINE ($x10^6$/ml)	cell counter
lab B, R	2.463	2.573	CellDyn Sapphire
Lab E	2.8	2.7	Coulter
lab J	3.2	2.9	Coulter Act Diff
lab K	2.7	2.4	Coulter Max M
lab M	2.72	2.66	Sysmex XE5000
Lab O	2.7	2.7	CellDyn Emerald
lab P	3.1	2.7	Coulter Act Diff
mean	**2.77 $x10^6$/ml**	**2.65 $x10^6$/ml**	
Total/30ml	**83.1 $x10^6$**	**79.5 $x10^6$**	
30% recovery	**24.9 $x10^6$**	**23.9 $x10^6$**	

Accuracy of cell counting was significantly improved in this QA round. Laboratories B and E may have miscalculated cell number or sample volume in some of their specimens, resulting in overestimation of the PBMC count, giving an apparent reduced fractionation recovery and excess thaw recovery. These out-of-range results when combined gave an acceptable absolute recovery (Table 1) demonstrating proficiency in fractionation and cryopreservation. However, the PBMC content in each ampoule remains a critical requirement in processing IVRN-sponsored blood samples. Comments on PBMC counting from the previous QA round report are repeated below:

> The accuracy of all PBMC recovery data depends on the accuracy of post-fractionation cell counting. The QA round was assessed using a Coulter Act Diff automated counter. Several labs used similar counters, and many labs also performed manual counting with a haemocytometer. In cases of high neutrophil or erythrocyte contamination resulting from abnormal or aged blood, an automated counter that can accurately quantify the major leukocyte subsets will be able to give a reasonably accurate PBMC count. Manual counting of the sample described above with a haemocytometer can result in overestimation of PBMC if the operator using a microscope with poor visual clarity and resolution cannot distinguish between the larger neutrophils and monocytes, and between erythrocytes and lymphocytes. Similarly, use of the whole blood count as the PBMC count ignores possible neutrophil contamination, thus overestimating the true PBMC count.

The other critical factor in accurate cell counting is to ensure that sampling technique and dilution is accurate. It is better to perform a small dilution or count neat from a moderately concentrated cell sample (5 – 10 ml) than to perform a large dilution from a highly concentrated low volume (1-2ml) sample. The sample should be evenly suspended without cell clumps. Cells must be evenly suspended when taking the sample for dilution, and equally important cells must be evenly suspended in the specimen tube in the cell counter. Human error in determining the cell sample volume, in making the dilution, in calculation of the final PBMC count, and uniform volume dispensing into cryovials, should also be scrutinised.

PBMC viability

The viability of all thawed PBMC specimens was >90% (Table 2), as determined by visual inspection of all samples in the presence of trypan blue, and confirmed as >95% by manual counting of selected specimens.

Relative and absolute PBMC recovery

The ability to recover sufficient PBMC during fractionation and for the end user to recover sufficient PBMC after thawing are key components of the QA assessment aimed at ensuring that laboratories entrusted with processing valuable blood samples can provide adequate PBMC samples to the study sponsor. Poor fractionation recovery is a waste of the sample. Low or excessively high post-thaw PBMC recovery is also a waste of sample according to the number of PBMC needed for an assay. Therefore, the IVRN QAP determined that fractionation recovery of >30% of PBMC from whole blood, and post-thaw recovery of PBMC ranging from 75% to 125% is required for a laboratory to be certified as competent for PBMC fractionation and cryopreservation. If recovery results outside the required range were the result of inaccurate counting, then the combined Absolute Recovery may indicate the true/overall PBMC recovery (eg. Lab E HIPO results).

Fractionation recovery, post-thaw recovery, and absolute recovery data are shown in Table 2. Results from each laboratory are represented by the same symbol between panels. The PBMC content of whole blood (Table 1) was considered a constant, and the thawing and counting of frozen PBMC was performed so as to minimise variation and hence was considered a virtual constant. Overestimation of the cell count relative to the whole blood PBMC count may result in a high fractionation recovery result (Figure 1a) but a correspondingly low post-thaw recovery (Figure 1b). However, absolute recovery of total PBMC (based on total vials frozen), relative to the whole blood count (Figure 1c), measures the skills of the scientist in producing PBMC, assuming no significant losses during thawing.

The cumulative trend in post-thaw viability and recovery over the past 10 QAP rounds is shown in Figure 2. The results from the 26th QA round show a marked improvement in post thaw recovery over previous rounds. The overall proficiency of the IVRN Tier 1 Laboratory Network in processing PBMC from day-old transported whole blood specimens is 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 both IVRN blood donors gave uniformly strong responses to the CEF peptide pool, with the expected variation in responses from individual local donors. All PBMC samples showed maximal stimulation in the presence of PMA and ionomycin (in excess of 5000 spots/million PBMC), and apart from one local donor specimen, all had low background responses in control medium, as expected from high quality functional PBMC.

Overall conclusions on performance in the 26th QA round

All labs achieved uniformly high viability results, and post thaw recovery results have improved significantly and show uniform high standard across all laboratories. This positions the IVRN Tier 1 Lab network at the highest of international standards for PBMC fractionation and cryopreservation, with highly capable laboratories around the country available for participation in clinical studies involving PBMC cryopreservation.

Thanks for your ongoing participation in the IVRN PBMC processing QAP, and contributing to the 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.

26th IVRN QAP report was produced by Dr Wayne Dyer, on behalf of the IVRN Executive.

Table 1. 26th IVRN Single Donor QA Round: PBMC Fractionation Recovery, Viability, Viable Recovery and Function.

			IVRN Tier 1 laboratory 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	11/11/15	30	10	2	20	24.1	12.909	129.1	31.1	>90	2	870	>5000	no	high	yes	pass
	HIV neg	11/11/15	30	10	2	20	25.2	12.388	123.9	31.2	>90	11	770	>5000	no	yes	yes	
	local donor	12/11/15	30	9.62	3	28.86	35.9	11.282	117.3	42.1	>90	18	740	>5000	yes	yes	yes	
C	HIV-pos	11/11/15	15	7.8	3	23.4	56.3	6.951	89.1	50.2	>90	2	1670	>5000	yes	yes	yes	pass
	HIV neg	11/11/15	15	8.8	3	26.4	66.4	7.455	84.7	56.2	>90	5	1600	>5000	yes	yes	yes	
	local donor	12/11/15	7	8	3	24	90.2	8.847	110.6	99.8	>90	6	210	>5000	yes	yes	yes	
E	HIV-pos	11/11/15	15	8.25	1	8.25	19.9	14.880	180.4	35.9	>90	0	1670	>5000	no	high	yes	pass
	HIV neg	11/11/15	15	8.5	2	17	42.8	10.395	122.3	52.3	>90	1	1700	>5000	yes	yes	yes	
	local donor	12/11/15	27	14.1	2	28.2	37.3	14.895	105.6	39.4	>90	63	0	>5000	yes	yes	high control	
F	HIV-pos	11/11/15	30	10	7	70	84.3	7.880	78.8	66.4	>90	2	1560	>5000	yes	yes	yes	pass
	HIV neg	11/11/15	30	9.4	7	65.8	82.8	6.881	73.2	60.6	>90	8	1450	>5000	yes	no	yes	
	local donor	12/11/15	27	9.4	4	37.6	OK	7.872	83.7	NA	>90	27	2140	>5000	yes	yes	yes	
J	HIV-pos	11/11/15	30	9.3	6	55.8	67.1	6.867	73.8	49.5	>90	2	1260	>5000	yes	no	yes	pass
	HIV neg	11/11/15	30	8.83	6	52.98	66.6	7.688	87.1	58.0	>90	1	1450	>5000	yes	yes	yes	
	local donor	12/11/15	30	6.25	2	12.5	69.4	4.923	78.8	54.7	>90	7	340	>5000	yes	yes	yes	
K	HIV-pos	11/11/15	30	7.5	6	45	54.2	6.429	85.7	46.4	>90	1	1340	>5000	yes	yes	yes	pass
	HIV neg	11/11/15	30	7.3	6	43.8	55.1	6.448	88.3	48.7	>90	2	1370	>5000	yes	yes	yes	
	local donor	12/11/15	27	7	4	28	51.9	6.195	88.5	45.9	>90	4	0	>5000	yes	yes	yes	
M	HIV-pos	11/11/15	30	10	3.5	35	42.1	9.300	93.0	39.2	>90	1	1420	>5000	yes	yes	yes	pass
	HIV neg	11/11/15	30	10	2.8	28	35.2	7.712	77.1	27.1	>90	1	1190	>5000	yes	yes	yes	
	local donor	12/11/15	54	10	6.9	69	59.2	9.870	98.7	58.4	>90	0	120	>5000	yes	yes	yes	
O	HIV-pos	11/11/15	30	8.75	4	35	42.1	8.415	96.2	40.5	>90	2	1090	>5000	yes	yes	yes	pass
	HIV neg	11/11/15	30	9	5	45	56.6	10.374	115.3	65.3	>90	2	1130	>5000	yes	yes	yes	
	local donor	12/11/15	27	9.17	2	18.34	37.7	8.373	91.3	34.4	>90	0	360	>5000	yes	yes	yes	
P	HIV-pos	11/11/15	30	8.8	7	61.6	74.1	8.407	95.5	70.8	>90	2	1880	>5000	yes	yes	yes	pass
	HIV neg	11/11/15	30	8.95	7	62.65	78.8	8.883	99.3	78.2	>90	6	1670	>5000	yes	yes	yes	
	local donor	12/11/15	21	6.15	6	36.9	83.7	5.940	96.6	80.9	>90	3	10	>5000	yes	yes	yes	
R	HIV-pos	11/11/15	30	6.4	6	38.4	46.2	6.867	107.3	49.6	>90	2	590	>5000	yes	yes	yes	pass
	HIV neg	11/11/15	30	5.9	6	35.4	44.5	5.388	91.3	40.6	>90	2	1090	>5000	yes	yes	yes	
	local donor	12/11/15	15	6.8	4	27.2	68.9	6.331	93.1	64.1	>90	19	1020	>5000	yes	yes	yes	

Notes:

(1) Assessment criteria 1: The minimum required fractionation recovery was 30% of available PBMC. Total PBMC averaged 83.1 million PBMC/30ml blood from HIV-pos and 79.5 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) >542 & >751/10^6 PBMC (HIV-pos & -neg); control (mean +2SD) <3 & <11 spots/well (HIV-pos & -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 nunber of vials produced / total PBMC in whole blood sample.

Red shading indicate results that are outside the performance standards.

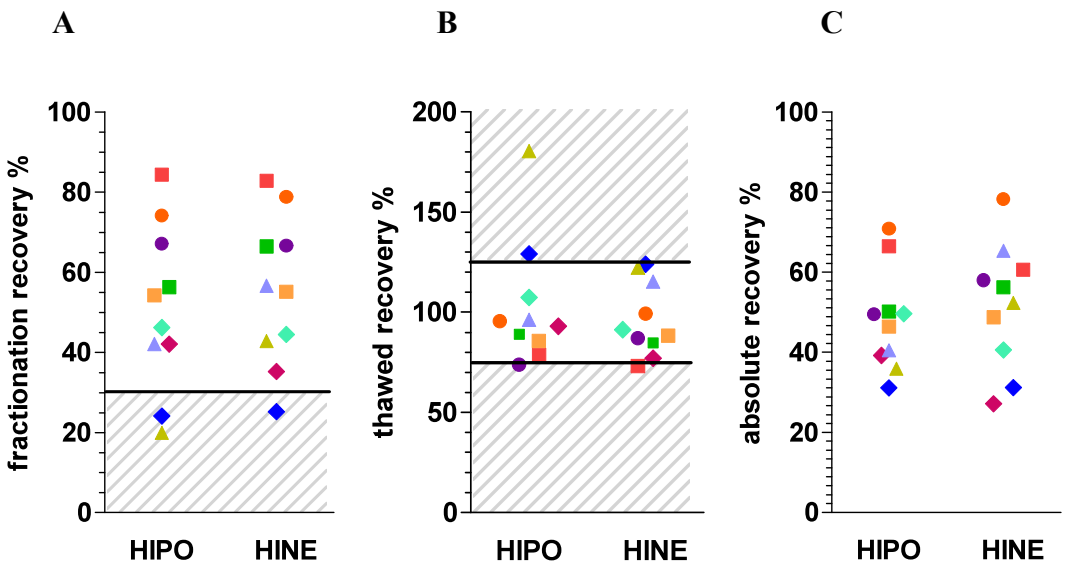

Figure 1. Comparison of relative vs. absolute recovery of PBMC showing (A) post fractionation recovery relative to laboratory cell count; (B) thawed PBMC recovery relative to laboratory cell count, and (C) absolute recovery of PBMC expressed as the % of the mean whole blood PBMC count. Shaded areas in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

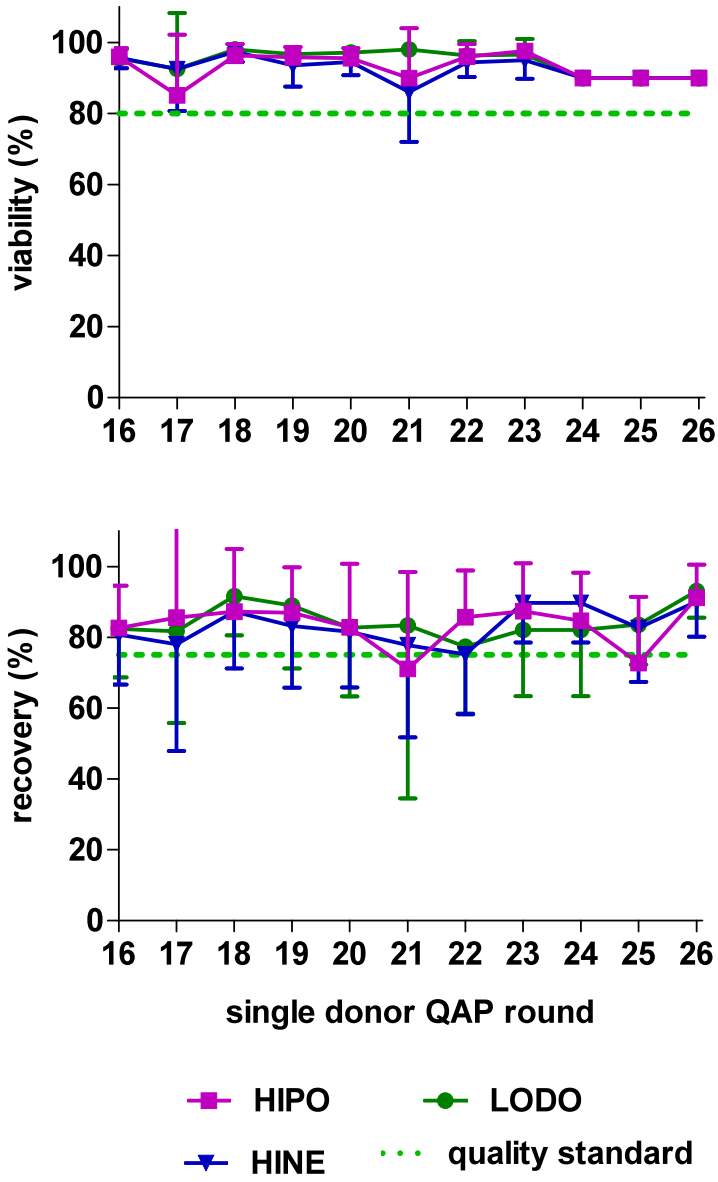

Figure 2. Cumulative trend in viability and post thaw recovery compared with the 10 previous QA rounds.
Mean and standard deviation; recovery results >100% were rounded down to a maximum recovery of 100%.

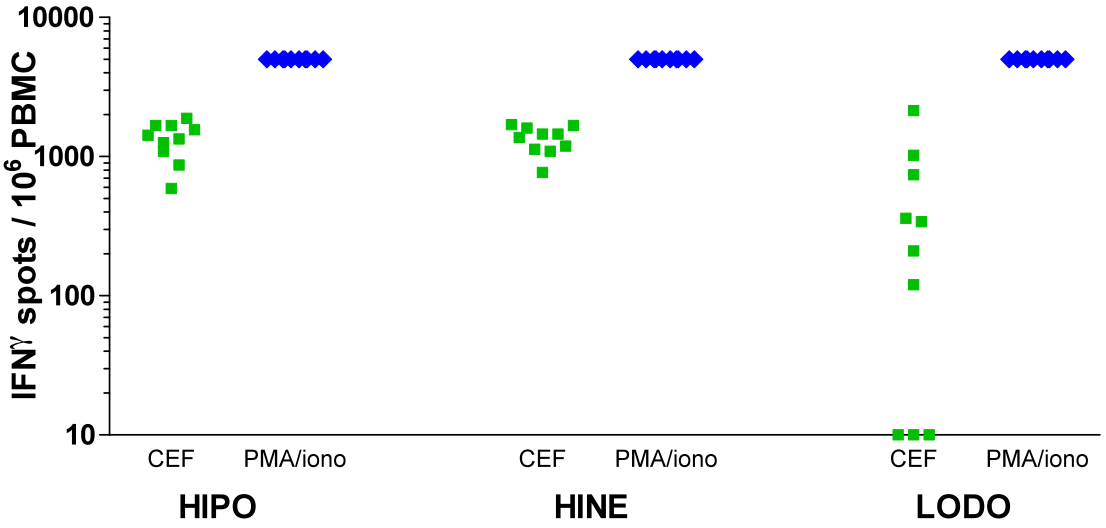

Figure 3. PBMC function results determined by IFN-γ ELISPOT. Antigen-specific responses were determined by stimulation and overnight culture with the CEF peptide pool, and maximal cytokine release with PMA + ionomycin.

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
	24th round	25th round	26th round	(passed 2 of 3 QAP rounds)
B	yes	no	yes	**Certified**
C	yes	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	no	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.