Report on the 31st IVRN PBMC cryopreservation QA round, May 2018

Blood assessed in this QA round was obtained from the IVRN donors on 14th May 2018 and transported to participating laboratories for processing the following morning along with a freshly obtained local blood sample. Cryopreserved PBMC specimens were assessed on 11th June.

PBMC fractionation recovery

The total number of PBMC available for fractionation in the IVRN blood samples was calculated from full blood differential counts. Counts from fresh blood samples taken soon after collection were compared with counts from 24 hour old specimens provided by labs on the day the QA round was performed. The average PBMC content of the IVRN blood samples counted on the day of the QA exercise was similar to the fresh blood count (Table 1). All laboratories achieved at least 30% fractionation recovery from the IVRN blood samples (Table 2). The mean fractionation efficiency for all specimens processed was 58%, indicating highly efficient recovery of PBMC.

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

Laboratory	HIPO ($x10^6$/29ml)	HINE ($x10^6$/29ml)	cell counter
fresh blood	54.52	73.59	Coulter Act Diff
lab B	50.79	70.5	Sysmex XN20
lab J	56.07	78.24	Coulter Act Diff
lab K	53.75	61.75	Coulter LH500
lab O	62.5	83.66	CellDyn Emerald
lab P	75.01	102.4	Coulter Act Diff
lab R	51.32	70.5	Sysmex XN20
24 hr bloods (average)	58.24 $x10^6$	77.84 $x10^6$	24 hr bloods (average)

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

In order to maximise return of PBMC from precious clinical specimens, the requirement to dispense an exact number of PBMC within a tight band of numerical accuracy is important. Incorrect cell counting may result in an inverse association between high fractionation recovery and low post-thaw recovery (eg. Lab F & Lab P local donor), and conversely an underestimation of cells may result in a low fractionation recovery but an excess post thaw recovery (eg. lab J). This inverse correlation between apparent fractionation recovery and thawed recovery can be seen in Figure 1 showing low fractionation recoveries (Fig 1A) and correspondingly high post thaw recoveries (Fig 1B). However, the absolute recovery of thawed PBMC, expressed as a percentage of PBMC in the fresh blood samples (Fig 1C) demonstrates a tight cluster between 40-65%. Therefore, all labs were able to fractionate and cryopreserve sufficient viable PBMC

The cumulative trend in viability and post-thaw recovery over the past 10 QAP rounds is shown in Figure 2, and demonstrates an overall improvement in post-thaw recovery seen in this QA round.

Functional analysis

The IFNγ ELISPOT assay was used to determine PBMC function, measuring 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). The same donors were used in this QA round as well as the previous QA round, and responses to the CEF peptide pool were again low, whereas 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). Background response in the presence of medium alone was low (except two specimens from Lab F, Table 2) and uniform strong response to PMA/ionomycin suggest that PBMC function was acceptable.

Overall conclusions on performance in the 31st QA round

The overall results from this QA round were the best since the 26th and 27th QA rounds, where post thaw recovery was at an all-time high. Unfortunately, one lab failed this QA round because of low post thaw recovery. The IVRN Tier 1 Lab network is assessed according to the highest of international standards for PBMC fractionation and cryopreservation. All labs achieved uniformly high viability results, and post-thaw recovery of PBMC continued to improve overall. The absolute recovery and function of PBMC suggests that all labs can fractionate and cryopreserve sufficient good quality PBMC from the available blood samples. 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.

31st IVRN QAP report was produced by Dr Wayne Dyer, on behalf of the IVRN Executive.

Table 2. 31st 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	fractionation [1] 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	14/5/18	30	9.03	3	27.09	46.5	10.406	115.2	53.6	>95	5	0	>5000	yes	yes	yes	
B	HIV neg	14/5/18	30	9.72	5	48.6	62.4	9.970	102.6	64.0	>95	2	20	>5000	yes	yes	yes	pass
	local donor	15/5/18	16	8.2	2	16.4	77.1	5.462	66.6	51.4	>95	1	0	>5000	yes	yes	yes	
	HIV-pos	14/5/18	30	9.1	5	45.5	78.1	7.410	81.4	63.6	>95	6	80	>5000	yes	yes	yes	
E	HIV neg	14/5/18	30	8.9	5	44.5	57.2	5.940	66.7	38.2	>95	6	0	>5000	yes	no	yes	pass
	local donor	15/5/18	27	9.8	3	29.4	54.4	7.944	81.1	44.1	>95	5	180	>5000	yes	yes	yes	
	HIV-pos	14/5/18	30	10	4	40	68.7	6.874	68.7	47.2	>95	60	0	>5000	yes	no	high control	
F	HIV neg	14/5/18	30	10	5	50	64.2	6.902	69.0	44.3	>95	12	100	>5000	yes	no	yes	fail
	local donor	15/5/18	27	10	5	50	NA	5.826	58.3	NA	>95	38	40	>5000	yes	no	yes	
	HIV-pos	14/5/18	30	10.5	2	21	36.1	12.714	121.1	43.7	>95	4	0	>5000	yes	yes	yes	
J	HIV neg	14/5/18	30	12	2	24	30.8	15.744	131.2	40.5	>95	3	20	>5000	yes	no	yes	pass
	local donor	15/5/18	12	6.4	1	6.4	30.4	7.410	115.8	35.2	>95	2	180	>5000	yes	yes	yes	
	HIV-pos	14/5/18	30	4.2	6	25.2	43.3	5.346	127.3	55.1	>95	8	70	>5000	yes	no	yes	
K	HIV neg	14/5/18	30	5.3	8	42.4	54.5	4.970	93.8	51.1	>95	3	30	>5000	yes	yes	yes	pass
	local donor	15/5/18	27	6.8	5	34	59.0	5.928	87.2	51.4	>95	9	630	>5000	yes	yes	yes	
	HIV-pos	14/5/18	30	8.75	4	35	60.1	7.433	84.9	51.1	>95	5	50	>5000	yes	yes	yes	
O	HIV neg	14/5/18	30	8.6	6	51.6	66.3	7.455	86.7	57.5	>95	1	40	>5000	yes	yes	yes	pass
	local donor	15/5/18	14	8.25	4	33	51.2	7.448	90.3	46.2	>95	0	1940	>5000	yes	yes	yes	
	HIV-pos	14/5/18	30	10	4	40	68.7	9.850	98.5	67.7	>95	8	0	>5000	yes	yes	yes	
P	HIV neg	14/5/18	30	10	6	60	77.1	7.968	79.7	61.4	>95	0	40	>5000	yes	yes	yes	pass
	local donor	15/5/18	18	10	3	30	94.4	7.433	74.3	70.2	>95	1	1320	>5000	yes	no	yes	
	HIV-pos	14/5/18	30	5.7	5	28.5	48.9	5.429	95.2	46.6	>95	9	0	>5000	yes	yes	yes	
R	HIV neg	14/5/18	30	8	6	48	61.7	6.944	86.8	53.5	>95	2	0	>5000	yes	yes	yes	pass
	local donor	15/5/18	18	5	2	10	41.8	4.925	98.5	41.2	>95	0	0	>5000	yes	yes	yes	

Notes: **(1) Assessment criteria 1:** The minimum required fractionation recovery was 30% of available PBMC, which averaged 58.24 million PBMC/30ml blood from the HIV-pos and 77.84 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, counted 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 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 (mean +2SD) <52 & <12 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.

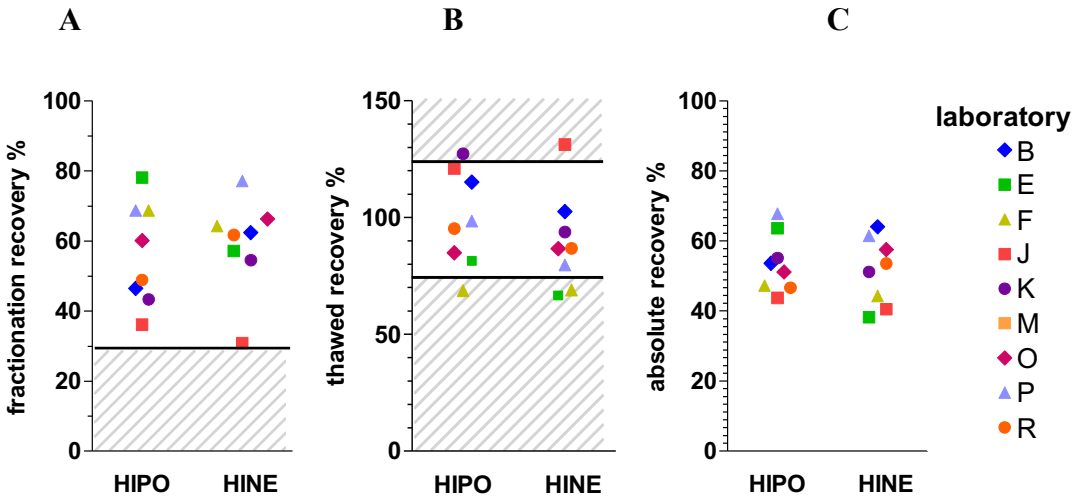

Figure 1. Comparison of relative vs. absolute recovery of PBMC showing (A) post fractionation recovery relative to laboratory cell count; (B) thawed PBMC recovery relative to laboratory cell count, and (C) absolute recovery of PBMC (total thawed PBMC x number of vials) expressed as the % of the mean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

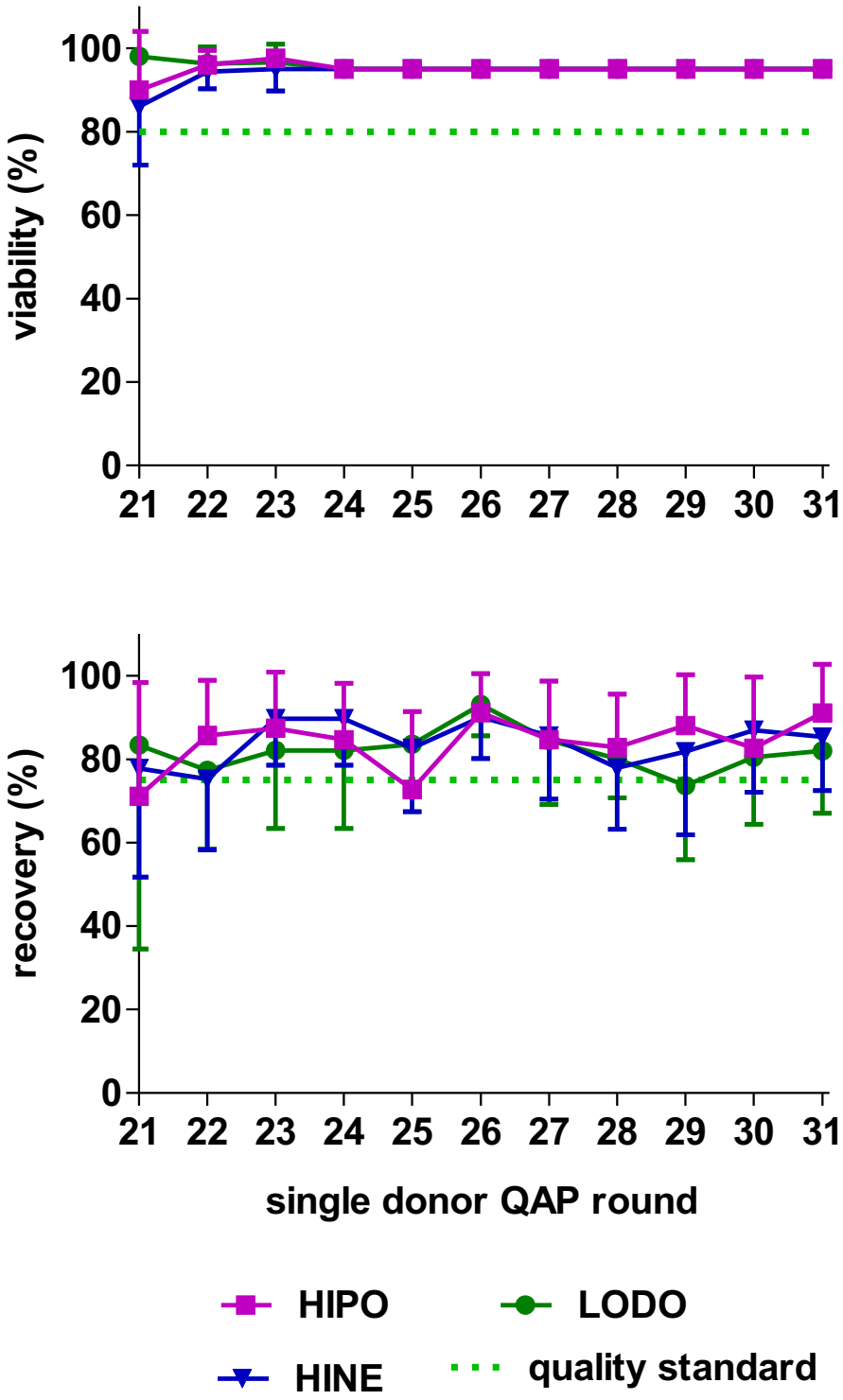

Figure 2. Cumulative trend in viability and post thaw recovery compared with the 10 previous QA rounds.
Mean and standard deviation; post thaw recovery results >100% were reported as 100%.

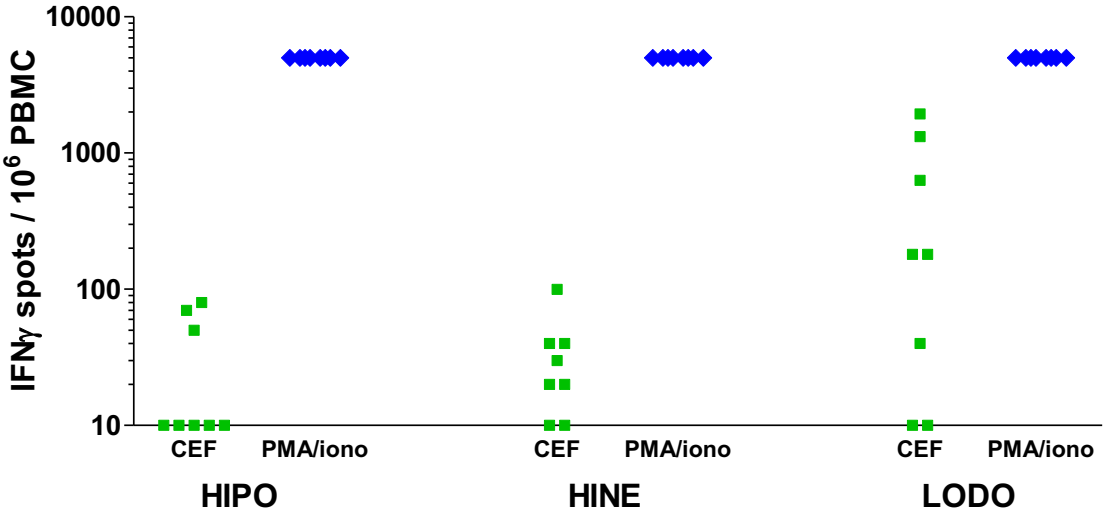

Figure 3. PBMC function results determined by IFN-γ ELISPOT. Antigen-specific responses were determined by stimulation and overnight culture with the CEF peptide pool, and maximal cytokine release with PMA + ionomycin.

Table 3. Current certification status of Tier 1 labs.

lab code	Performed adequately over the previous QAP rounds? (all 4 quality standards met in at least one PBMC specimen)			current status
	29th round	30th round	31st round	(passed 2 of 3 QAP rounds)
B	pass	fail	pass	**Certified**
E	pass	pass	pass	**Certified**
F	fail	pass	fail	**Certified – Under Review**
J	fail	pass	pass	**Certified**
K	pass	fail	pass	**Certified**
M	pass	pass	NA	**Certified**
O	pass	pass	pass	**Certified**
P	pass	pass	pass	**Certified**
R	pass	pass	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. 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.