Report on the 32nd IVRN PBMC cryopreservation QA round, Dec 2018

Blood was collected from the IVRN donors for this QA round on 12th Dec 2018, the QA exercise took place on 13th Dec, cryopreserved PBMC specimens were returned on 17th Dec, and QA assessments took place during Jan 2019.

The next QA round will take place on Tuesday 21st May 2019.

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 (Table 1). The average PBMC content of IVRN blood samples counted on the day of the QA exercise was used to calculate fractionation recovery for the IVRN blood samples (Table 2). The mean fractionation efficiency was 56%, indicating highly efficient recovery of PBMC.

Table 1. Total PBMC in 30ml whole blood samples for 32nd QA round, reported from each lab on the day of QAP processing.

Laboratory	HIPO ($x10^6$/29ml)	HINE ($x10^6$/29ml)	cell counter
fresh blood	50.34	61.99	Coulter Act Diff
lab B, R	57.03	53.67	Sysmex XN20
lab J	51.48	53.4	Coulter Act Diff
lab K	48.51	42.12	Coulter LH500
lab M	52.86	55.38	
lab O	63.66	64.68	CellDyn Emerald
lab P	52.17	61.38	Coulter Act Diff
lab T	NA	53.79	Coulter DxH520
24hr bloods (average)	54.3 $x10^6$	54.9 $x10^6$	

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. Inaccurate cell counting is demonstrated by an inverse association between fractionation recovery and post-thaw recovery (Figure 1). Two laboratories failed this QA round because of low post-thaw recovery (Fig 1B- labs K & M), whereas the reported fractionation recovery for these specimens was high (Fig 1A). The absolute recovery of thawed PBMC, expressed as a percentage of PBMC in the fresh blood samples (Fig 1C) demonstrates a tight cluster between 30-50%, suggesting overall proficiency in provision of viable PBMC from the respective IVRN blood samples.

The cumulative trend in viability and post-thaw recovery over the past 10 QAP rounds is shown in Figure 2.

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 HIV-pos donors was used in this QA round as in the previous QA round, and responses to the CEF peptide pool were again low. Data from the HIV-neg donor PBMC were also tightly clustered, 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 in the IVRN specimens was acceptable.

Overall conclusions on performance in the 32nd QA round

The IVRN Tier 1 Lab network is assessed according to the highest of international standards for PBMC fractionation and cryopreservation, which includes a high expectation for actual post-thaw recovery. All labs achieved uniformly high viability results, however two labs failed this QA round because of low post thaw recovery. 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). 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.

32nd IVRN QAP report was produced by Dr Wayne Dyer, on behalf of the IVRN Executive.

Table 2. 32nd 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/recover	[4] Adequate response in function assays	[5] overall result
B	HIV-pos	12/12/2018	30	8.85	3	26.55	48.9	8.296	93.7	45.8	93	7	30	>5000	yes	yes	yes	
	HIV neg	12/12/2018	30	9.41	3	28.23	51.4	8.228	87.4	45.0	>95	3	150	>5000	yes	yes	yes	pass
	local donor	13/12/2018	18	6.05	2	12.1	49.7	4.500	74.4	37.0	>95	3	3180	>5000	yes	no	yes	
E	HIV-pos	12/12/2018	30	8.87	2	17.74	32.7	8.449	95.3	31.1	>95	10	20	>5000	yes	yes	yes	
	HIV neg	12/12/2018	30	8	2	16	29.1	7.904	98.8	28.8	>95	4	150	>5000	no	yes	yes	pass
	local donor	13/12/2018	27	9.25	2	18.5	48.9	9.367	101.3	49.5	>95	14	1630	>5000	yes	yes	yes	
F	HIV-pos	12/12/2018	30	12	3	36	66.3	8.883	74.0	49.1	>95	27	50	>5000	yes	no	no	
	HIV neg	12/12/2018	30	12	2	24	43.7	10.813	90.1	39.4	>95	72	10	>5000	yes	yes	no	pass
	local donor	13/12/2018	36	11.8	5	59	NA	10.353	87.7	NA	>95	24	0	>5000	yes	yes	yes	
J	HIV-pos	12/12/2018	30	10	3.6	36	66.3	6.776	67.8	44.9	92	16	0	>5000	yes	no	yes	
	HIV neg	12/12/2018	30	10	3	30	54.6	8.766	87.7	47.9	>95	3	120	>5000	yes	yes	yes	pass
	local donor	13/12/2018	25	9.7	2	19.4	49.9	9.910	102.2	51.0	97	4	890	>5000	yes	yes	yes	
K	HIV-pos	12/12/2018	30	7.8	5	39	71.8	4.915	63.0	45.3	>95	12	0	>5000	yes	no	yes	
	HIV neg	12/12/2018	30	7.6	5	38	69.2	4.930	64.9	44.9	97	2	170	>5000	yes	no	yes	fail
	local donor	13/12/2018	27	6.75	4	27	63.5	4.905	72.7	46.1	>95	1	830	>5000	yes	no	yes	
M	HIV-pos	12/12/2018	30	6.08	6	36.48	67.2	4.000	65.8	44.2	>95	12	0	>5000	yes	no	yes	
	HIV neg	12/12/2018	30	5.59	5	27.95	50.9	4.000	71.6	36.4	>95	1	40	>5000	yes	no	yes	fail
	local donor	13/12/2018	60	12.5	6	75	56.6	8.424	67.4	38.1	>95	4	290	>5000	yes	no	yes	
O	HIV-pos	12/12/2018	30	11.7	3	35.1	64.6	7.856	67.1	43.4	>95	9	40	>5000	yes	no	yes	
	HIV neg	12/12/2018	30	10.5	3	31.5	57.4	6.435	61.3	35.2	>95	0	250	>5000	yes	no	yes	pass
	local donor	13/12/2018	18	9.3	3	27.9	58.7	7.365	79.2	46.5	>95	1	770	>5000	yes	yes	yes	
P	HIV-pos	12/12/2018	30	10	4	40	73.7	8.356	83.6	61.6	>95	19	0	>5000	yes	yes	yes	
	HIV neg	12/12/2018	30	10	4	40	72.9	7.896	79.0	57.5	>95	2	120	>5000	yes	yes	yes	pass
	local donor	13/12/2018	22.5	8	3	24	70.0	7.463	93.3	NA	>95	4	1010	>5000	yes	yes	yes	
R	HIV-pos	12/12/2018	26	5.9	3	17.7	32.6	4.915	83.3	27.2	91	6	40	>5000	yes	yes	yes	
	HIV neg	12/12/2018	27	7.17	3	21.51	39.2	5.946	82.9	32.5	>95	2	100	>5000	yes	yes	yes	pass
	local donor	13/12/2018	18	5.2	2	10.4	42.8	4.000	76.9	32.9	98	3	3520	>5000	yes	yes	yes	
T	NA HIV neg	12/12/2018	30	7.58	4	30.32	55.2	5.886	77.7	42.9	>95	1	250	>5000	yes	yes	yes	pass
	local donor	13/12/2018	32	7.58	4	30.32	77.8	6.888	90.9	70.7	>95	1	0	>5000	yes	yes	yes	

Notes: **(1) Assessment criteria 1:** The minimum required fractionation recovery was 30% of available PBMC, which averaged 54.3 million PBMC/30ml blood from the HIV-pos and 54.9 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) <26 & <51 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.

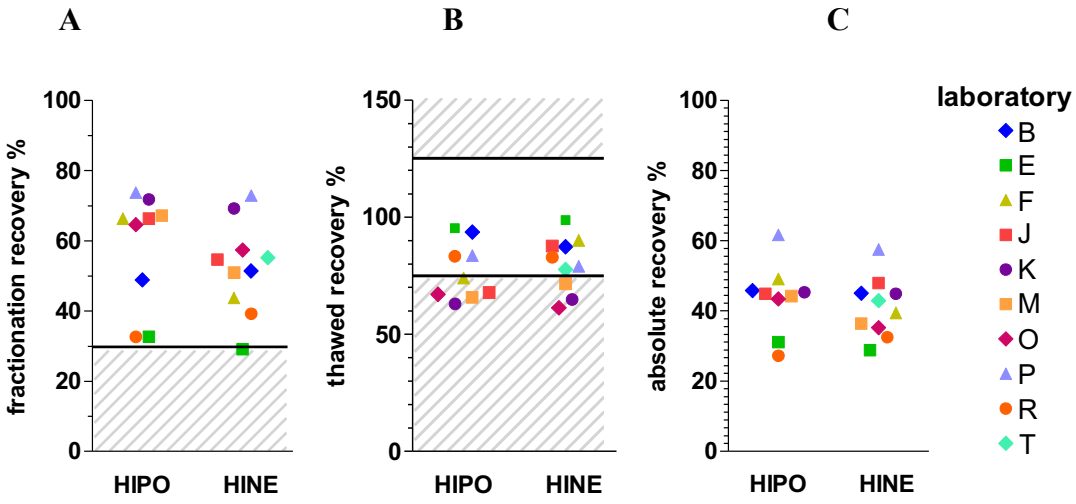

Figure 1. Comparison of relative vs. absolute recovery of PBMC showing (A) post fractionation recovery relative to laboratory cell count; (B) thawed PBMC recovery relative to laboratory cell count, and (C) absolute recovery of PBMC (total thawed PBMC x number of vials) expressed as the % of the mean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

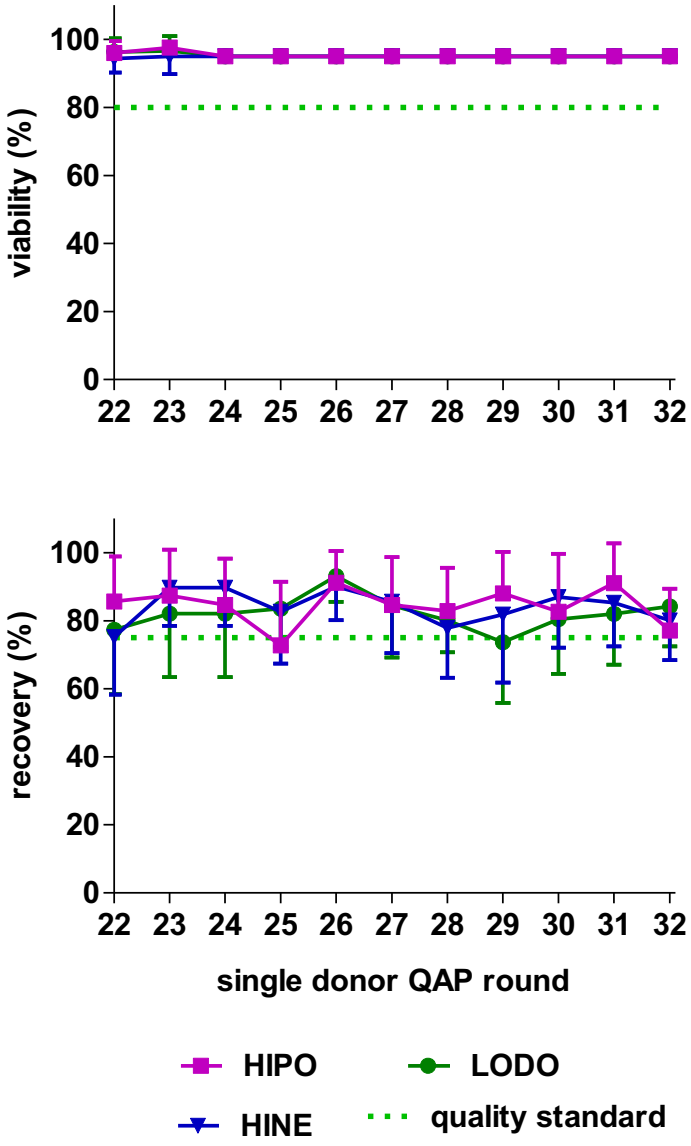

Figure 2. Cumulative trend in viability and post thaw recovery compared with the 10 previous QA rounds.
Mean and standard deviation; post thaw recovery results >100% were reported as 100%.

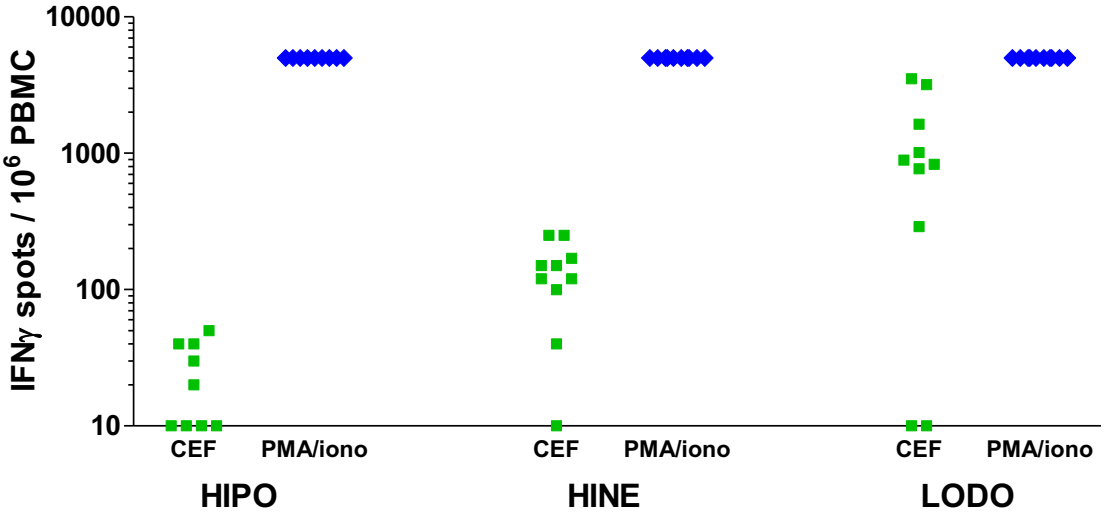

Figure 3. PBMC function results determined by IFN-γ ELISPOT. Antigen-specific responses were determined by stimulation and overnight culture with the CEF peptide pool, and maximal cytokine release with PMA + ionomycin.

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
	30th round	31st round	32nd round	(passed 2 of 3 QAP rounds)
B	fail	pass	pass	**Certified**
E	pass	pass	pass	**Certified**
F	pass	fail	pass	**Certified**
J	pass	pass	pass	**Certified**
K	fail	pass	fail	**Certified – Under Review**
M	pass	NA	fail	**Certified – Under Review**
O	pass	pass	pass	**Certified**
P	pass	pass	pass	**Certified**
R	pass	pass	pass	**Certified**
T			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.