Report on the 30th IVRN PBMC cryopreservation QA round, Nov 2017

Blood was taken from the IVRN donors on 21st November 2017 and transported to participating laboratories for processing the following morning along with a freshly obtained local blood sample. Cryopreserved PBMC specimens were assessed on 26th November.

Specimen labels and dispensing

Participating laboratories are not penalised for deviations in specimen labelling or dispensing in the QAP, but please consider the following points:

- Label vials with the specimen collection date, not the processing date (eg. **labs F & M**).
- It is considered wasteful to dispense more than 10 x 10^6 PBMC per vial, because an assay may require only a small number of PBMC; eg. **Lab J**, instead of dividing 32 x 10^6 PBMC into two vials of 16 x 10^6 PBMC, a better option would be to divide this number of PBMC between 3 or 4 vials.

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). Two results (shaded) were considered outliers and not used to calculate the average whole blood PBMC count. All laboratories achieved at least 30% fractionation recovery from the IVRN blood samples (Table 2). The mean fractionation efficiency for all specimens processed was 61%, 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	57.97	85.84	Coulter Act Diff
lab B, R	60.67	95.58	Sysmex XN20
Lab E	57.97	85.84	Coulter
lab J	60.42	98.28	Coulter Act Diff
lab K	62.12	90.55	Coulter LH500
lab M	51.3	81.9	Sysmex XE5000
lab O	37.44	94.5	CellDyn Emerald
lab P	70.64	138.57	Coulter Act Diff
24 hr bloods (average)	60.52 x10^6	91.11 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. One specimen from Lab B had a few dead cells, and viability confirmed by manual counting was 91% (Table 2).

In order to maximise return of PBMC from precious clinical specimens, the requirement for dispensing an exact number of PBMC within a tight band of numerical accuracy is important. Overestimation or incorrect cell counting may result in an inverse association between high

fractionation recovery and low post-thaw recovery of PBMC. This association was not observed as frequently as in previous QA rounds. Examples from this QA round include HINE PBMC from **Lab B** and HIPO PBMC from **Lab F** (Table 2), and illustrated in Figure 1 showing abnormally high fractionation recoveries (Fig 1A) and correspondingly low post thaw recoveries (Fig 1B). **Labs B and F** counted PBMC manually in a haemocytometer. Interestingly, **Lab B** also used an automated counter, which gave higher counts than the haemocytometer counts, and use of the automated counts would have resulted in an overall Pass for **Lab B** in this QA round.

By way of reminder, please check the fresh blood counts, which are e-mailed to each lab before the QA exercise. If an unexpectedly high post-fractionation PBMC recovery is obtained, this should be confirmed in an automated cell counter if the first count was obtained from a haemocytometer.

The cumulative trend in viability and post-thaw recovery over the past 10 QAP rounds is shown in Figure 2, demonstrating a small improvement in post-thaw recovery 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 PBMC from both donors did not respond to the CEF peptide pool, whereas responses from individual local donors varied from undetectable to very strong, as expected. All PBMC samples showed maximal stimulation in the presence of PMA and ionomycin (in excess of 5000 spots/million PBMC). We know from previous QA rounds that there is considerable donor variability in the response to CEF peptides. Previous inclusion of freshly processed IVRN donor PBMC in the ELISPOT assay did not result in higher responses than from 24 hour old processed PBMC. Therefore, although CEF responses from the IVRN donor PBMC were negative again, overall low background (except specimens from **Lab K)** and uniform strong response to PMA/ionomycin suggest that PBMC function was acceptable.

Overall conclusions on performance in the 30th QA round

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, whereas recovery of PBMC was variable between labs, which appeared to be associated with cell counting issues. The absolute recovery and function response 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.

30th IVRN QAP report was produced by Dr Wayne Dyer, on behalf of the IVRN Executive.

Table 2. 30th 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	net spots/10^6 PBMC PMA/Iono	[1] Adequate PBMC fractionated	Adequate viability/recovery	[4] Adequate response in function assays	[5] overall result
	HIV-pos	21/11/17	30	9.5	4	38	63.5	5.385	56.7	36.0	91	4	10	>5000	yes	no	yes	
B	HIV neg	21/11/17	30	10.2	7	71.4	77.8	6.587	64.6	50.2	>95	2	30	>5000	yes	no	yes	fail
	local donor	21/11/17	15	10	1	10	24.1	5.826	58.3	14.0	>95	53	0	>5000	no	no	yes	
	HIV-pos	21/11/17	30	8.666	3	25.998	43.5	9.830	113.4	49.3	>95	7	0	>5000	yes	yes	yes	
E	HIV neg	21/11/17	30	9.37	6	56.22	61.3	9.386	100.2	61.4	>95	1	40	>5000	yes	yes	yes	pass
	local donor	22/11/17	30	9.46	7	66.22	57.3	8.449	89.3	51.2	>95	3	190	>5000	yes	yes	yes	
	HIV-pos	22/11/17	30	10	5	50	83.6	7.358	73.6	61.5	>95	36	0	>5000	yes	no	yes	
F	HIV neg	22/11/17	30	10	6	60	65.4	9.890	98.9	64.7	>95	11	0	>5000	yes	yes	yes	pass
	local donor	22/11/17	18	10	3	30	NA	6.902	69.0	NA	>95	34	1180	>5000	yes	no	yes	
	HIV-pos		30	16	2	32	53.5	12.779	79.9	42.7	>95	7	30	>5000	yes	yes	yes	
J	HIV neg		30	16	2	32	34.9	11.374	71.1	24.8	>95	1	50	>5000	yes	no	yes	pass
	local donor	22/11/17	30	16	2	32	52.9	12.805	80.0	42.3	>95	6	920	>5000	yes	yes	yes	
	HIV-pos	21/11/17	30	9.5	4	38	63.5	5.374	56.6	35.9	>95	54	0	>5000	yes	no	no	
K	HIV neg	21/11/17	30	7.8	7	54.6	59.5	5.340	68.5	40.7	>95	100	0	>5000	yes	no	no	fail
	local donor	22/11/17	27	6	5	30	52.2	3.500	58.3	30.5	>95	317	0	>5000	yes	no	high	
	HIV-pos	22/11/17	30	6.2	5	31	51.8	8.829	142.4	73.8	>95	3	0	>5000	yes	no	yes	
M	HIV neg	22/11/17	30	11.1	6	66.6	72.6	15.270	137.6	99.8	>95	1	20	>5000	yes	no	yes	pass
	local donor	22/11/17	50	7.5	6	45	61.6	8.356	111.4	68.6	>95	242	50	>5000	yes	yes	high	
	HIV-pos	21/11/17	30	10.2	5	51	85.2	9.339	91.6	78.0	>95	11	0	>5000	yes	yes	yes	
O	HIV neg	21/11/17	30	10.7	7	74.9	81.6	9.443	88.3	72.0	>95	2	50	>5000	yes	yes	yes	pass
	local donor	21/11/17	15.5	10	3	30	93.6	7.888	78.9	73.8	>95	21	60	>5000	yes	yes	yes	
	HIV-pos	21/11/17	30	7.9	5	39.5	66.0	7.410	93.8	61.9	>95	8	10	>5000	yes	yes	yes	
P	HIV neg	21/11/17	30	8.24	7	57.68	62.9	8.892	107.9	67.8	>95	2	20	>5000	yes	yes	yes	pass
	local donor	22/11/17	17	8.02	3	24.06	NA	9.481	118.2	NA	>95	4	320	>5000	yes	yes	yes	
	HIV-pos	21/11/17	30	7	5	35	58.5	6.412	91.6	53.6	>95	7	0	>5000	yes	yes	yes	
R	HIV neg	21/11/17	30	8.24	8	65.92	71.8	7.455	90.5	65.0	>95	1	20	>5000	yes	yes	yes	pass
	local donor	21/11/17	15	5	3	15	36.1	4.500	90.0	32.5	>95	39	0	>5000	yes	yes	yes	

Notes: **(1) Assessment criteria 1:** The minimum required fractionation recovery was 30% of available PBMC, which averaged 60.52 million PBMC/30ml blood from the HIV-pos and 91.11 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) <51 & <76 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: background spots from these local donor PBMC are very high.

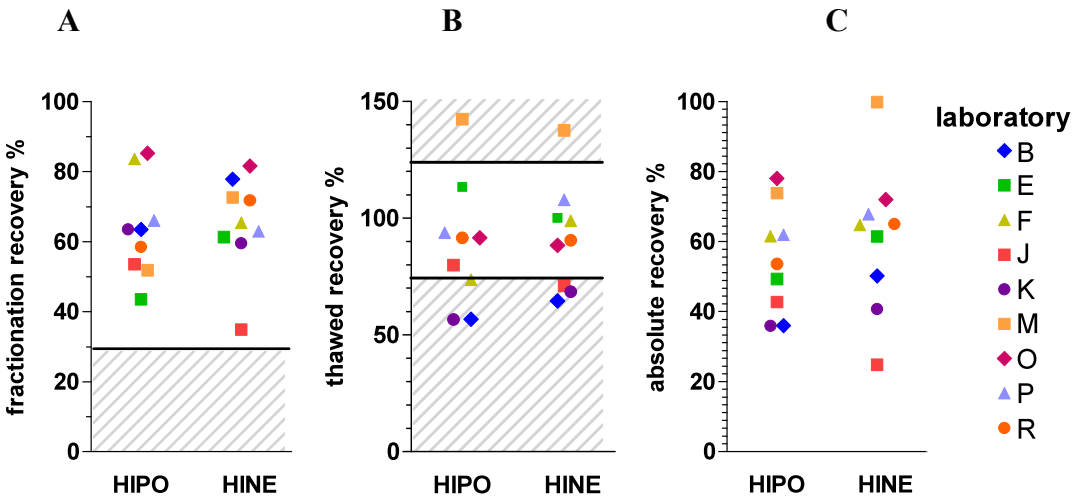

Figure 1. Comparison of relative vs. absolute recovery of PBMC showing (A) post fractionation recovery relative to laboratory cell count; (B) thawed PBMC recovery relative to laboratory cell count, and (C) absolute recovery of PBMC (total thawed PBMC x number of vials) expressed as the % of the mean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

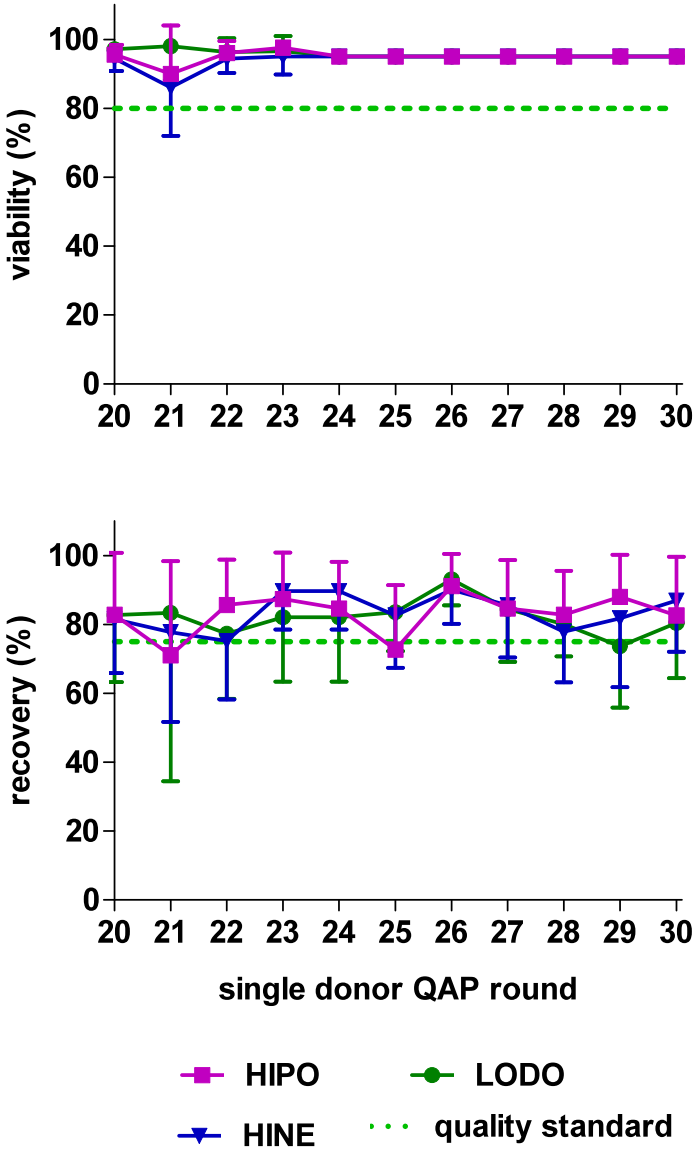

Figure 2. Cumulative trend in viability and post thaw recovery compared with the 10 previous QA rounds.
Mean and standard deviation; recovery results >100% were rounded down to a maximum recovery of 100%.

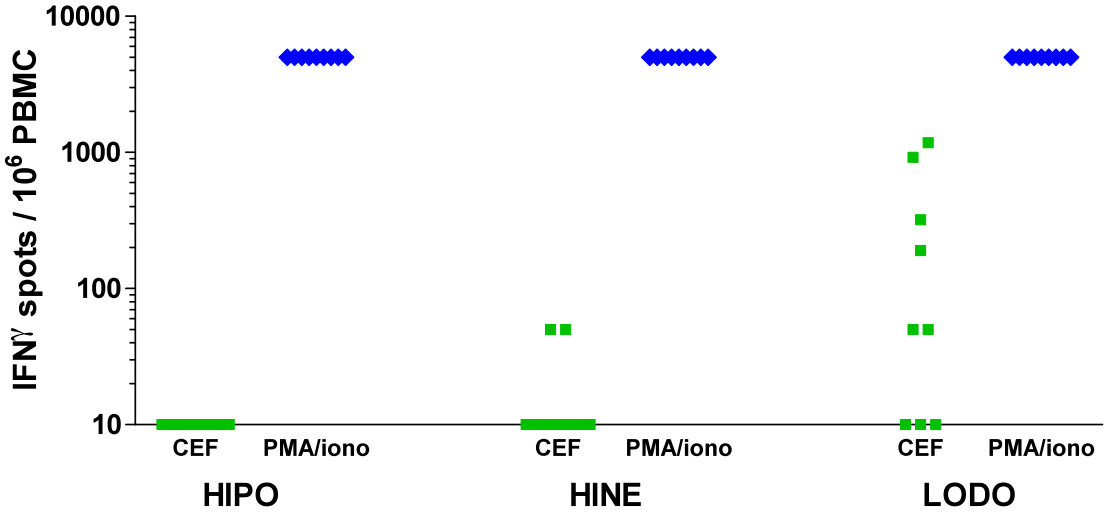

Figure 3. PBMC function results determined by IFN-γ ELISPOT. Antigen-specific responses were determined by stimulation and overnight culture with the CEF peptide pool, and maximal cytokine release with PMA + ionomycin.

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
	28th round	29th round	30th round	(passed 2 of 3 QAP rounds)
B	fail	pass	fail *	**Certified***
E	pass	pass	pass	**Certified**
F	pass	fail	pass	**Certified**
J	pass	fail	pass	**Certified**
K	pass	pass	fail	**Certified**
M	pass	pass	pass	**Certified**
O	pass	pass	pass	**Certified**
P	pass	pass	pass	**Certified**
R	pass	pass	pass	**Certified**

* **Lab B** recovery data would have resulted in a Pass if the automated cell count was used instead of the manual PBMC count. The lab supervisor stated that manual counting was not the usual procedure, and therefore retention of Certified status was considered reasonable in this circumstance.

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.