Report on the 29th IVRN PBMC cryopreservation QA round, May 2017

Blood was taken from the IVRN donors on 23rd May 2017 for processing the following morning along with a freshly obtained local blood sample at each laboratory. Cryopreserved PBMC specimens were assessed on 4th and 5th June.

Specimen labelling

Specimen labelling is not part of the QAP assessment, but please consider the following points:

- Label vials with the specimen collection date, not the processing date (eg. labs F, M, P).
- Fill and label vials with the total PBMC content, not variable volumes of fixed concentration (labs B, J). Add the same number of PBMC to all vials from the same specimen.

The specimen processing sheet in the IVRN manual will be updated so that PBMC are recorded as number of vials X total PBMC/vial (section 10), and a space provided in the PBMC results box for the volume of the PBMC specimen as counted to be recorded.

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. Feedback from labs suggested that the total volume of blood supplied in the two 15ml tubes was closer to 29ml, not 30ml, and was therefore taken into account. 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 donor blood samples (Table 2). The mean fractionation efficiency for all specimens processed was 53%, suggesting highly efficient recovery of PBMC.

Table 1. Total PBMC in 29ml whole blood samples for 29th QA round.

Laboratory	HIPO ($x10^6$/29ml)	HINE ($x10^6$/29ml)	cell counter
fresh blood	76.53	68.96	Coulter Act Diff
lab B, R	80.13	67.12	Sysmex XN20
Lab E	76.53	68.69	Coulter
lab J	82.36	74.82	Coulter Act Diff
lab K	68.59	66.91	Coulter LH500
lab M	70.76	63.51	Sysmex XE5000
lab O	76.53	74.69	CellDyn Emerald
24 hr bloods (average)	75.82 $x10^6$	69.34 $x10^6$	
30% recovery of 24 hr blood	**22.75 $x10^6$**	**20.80 $x10^6$**	

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 two stained cells were present in a field of view. Two specimens with a few dead cells had viability counts of 83% and 94%, and some of these cells may have been dead granulocytes. All other specimens did not contain many stained cells and viability was defined as >95% (Table 2).

As in previous QA rounds, discrepancies in cell counting can result in an inverse association between fractionation recovery and apparent post-thaw recovery of PBMC. Figure 1 illustrates this relationship, showing abnormally high fractionation recoveries >90% from one lab (Fig 1A), with corresponding post thaw recovery below the 75% cut-off (Fig 1B). When combined, these out-of-range recoveries resulted in an acceptable absolute recovery of viable PBMC from these blood specimens (Figure 1C, and Table 2). However, in order to maximise return of PBMC from precious clinical specimens, the requirement for dispensing PBMC within a tight band of numerical accuracy is important. It is worth noting that PBMC from this lab were counted manually in a haemocytometer, and the lab scientist confirmed that there were many erythrocytes present in their PBMC, which may have artificially inflated the PBMC count if mistaken for lymphocytes. Please note that fresh blood counts are e-mailed to each lab before the QA exercise. Therefore, if a high post-fractionation PBMC recovery is obtained (ie. >70%), 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. There was a small improvement in post-thaw recovery in this QA round. The combined IVRN Tier 1 Laboratory Network therefore demonstrates ongoing proficiency in processing PBMC from day-old transported whole blood specimens.

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). In this QA round, PBMC from both the HIV+ and the HIV-neg donor 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, these functional results suggest that PBMC quality was good, supported by low spontaneous (background) IFN-γ production.

Overall conclusions on performance in the 28th 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.

29th IVRN QAP report was produced by Dr Wayne Dyer, on behalf of the IVRN Executive.

Table 2. 29th 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
	HIV-pos	23/05/17	29	12	2	24	31.7	10.385	86.5	27.4	>95	8	<25	>5000	yes	yes	yes	
B	HIV neg	23/05/17	29	14	2	28	40.4	11.350	81.1	32.7	>95	1	0	>5000	yes	yes	yes	pass
	local donor	24/05/17	16	8	2	16	50.2	3.500	43.8	22.0	>95	6	3600	>5000	yes	no	yes	
	HIV-pos	23/05/17	29	10.8	3	32.4	42.7	11.430	105.8	45.2	>95	6	0	>5000	yes	yes	yes	
E	HIV neg	23/05/17	29	9.8	3	29.4	42.4	9.470	96.6	41.0	>95	1	<30	>5000	yes	yes	yes	pass
	local donor	24/05/17	27	9	2	18	33.3	6.481	72.0	24.0	>95	6	510	>5000	yes	no	yes	
	HIV-pos	23/05/17	29	10	7	70	92.3	6.435	64.4	59.4	>95	43	0	>5000	yes	no	high background	
F	HIV neg	23/05/17	29	11	6	66	95.2	5.417	49.2	46.9	>95	7	0	>5000	yes	no	high background	fail
	local donor	24/05/17	27	9.3	4	37.2	NA	4.500	48.4	NA	>95	23	<30	>5000	yes	no	yes	
	HIV-pos	23/05/17	29	10	4.2	42	55.4	7.328	73.3	40.6	>95	14	0	>5000	yes	no	yes	
J	HIV neg	23/05/17	29	10	2.5	25	36.1	5.445	54.5	19.6	>95	3	<15	>5000	yes	no	yes	fail
	local donor	24/05/17	12	5	1.5	15	77.4	3.500	70.0	54.2	>95	1	550	>5000	yes	no	yes	
	HIV-pos	23/05/17	29	7.4	6	44.4	58.6	6.923	93.6	54.8	>95	13	0	>5000	yes	yes	yes	
K	HIV neg	23/05/17	29	9.4	5	47	67.8	6.429	68.4	46.4	>95	1	85	>5000	yes	no	yes	pass
	local donor	24/05/17	27	7.2	3	21.6	52.5	5.832	81.0	42.5	>95	8	475	>5000	yes	yes	yes	
	HIV-pos	23/05/17	29	10.63	5	53.15	70.1	11.290	106.2	74.5	83	10	0	>5000	yes	yes	yes	
M	HIV neg	23/05/17	29	10.79	4	43.16	62.2	14.380	133.3	83.0	94	2	<10	>5000	yes	no	yes	pass
	local donor	24/05/17	46	10.83	5	54.15	84.2	8.604	79.4	66.9	>95	6	340	>5000	yes	yes	yes	
	HIV-pos	23/05/17	29	8.7	6	52.2	68.8	7.403	85.1	58.6	>95	12	0	>5000	yes	yes	yes	
O	HIV neg	23/05/17	29	9.2	5	46	66.3	7.944	86.3	57.3	>95	0	55	>5000	yes	yes	yes	pass
	local donor	24/05/17	16	8	2	16	43.8	9.310	116.4	51.0	>95	6	0	>5000	yes	yes	yes	
	HIV-pos	23/05/17	29	8.9	4	35.6	47.0	8.380	94.2	44.2	>95	11	0	>5000	yes	yes	yes	
P	HIV neg	23/05/17	29	8.3	3	24.9	35.9	9.870	118.9	42.7	>95	1	<20	>5000	yes	yes	yes	pass
	local donor	24/05/17	18	5	2	10	31.8	4.500	90.0	28.6	>95	3	1130	>5000	yes	yes	yes	
	HIV-pos	23/05/17	29	6.76	8	54.08	71.3	6.448	95.4	68.0	>95	5	<25	>5000	yes	yes	yes	
R	HIV neg	23/05/17	29	5.5	7	38.5	55.5	5.483	99.7	55.4	>95	1	<40	>5000	yes	yes	yes	pass
	local donor	24/05/17	25	6.36	2	12.72	25.5	4.920	77.4	19.7	>95	14	3490	>5000	no	yes	yes	

Notes: **(1) Assessment criteria 1:** The minimum required fractionation recovery was 30% of available PBMC, which averaged 75.82 million PBMC/29ml blood from the HIV-pos and 69.34 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: 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) 0/10^6 PBMC (HIV+ & neg); control (mean +2SD) <36 & <6 spots/well (HIV+ & neg). Limit of detection was 50 spots/10^6 PBMC.

(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.

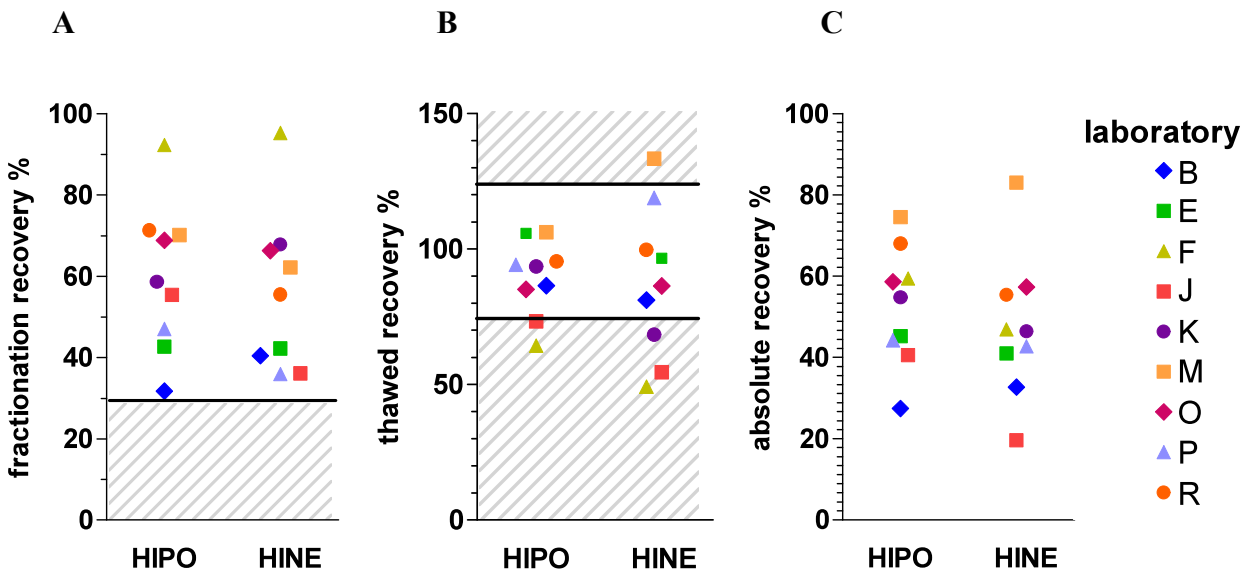

Figure 1. Comparison of relative vs. absolute recovery of PBMC showing (A) post fractionation recovery relative to laboratory cell count; (B) thawed PBMC recovery relative to laboratory cell count, and (C) absolute recovery of PBMC (total thawed PBMC x number of vials) expressed as the % of the mean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

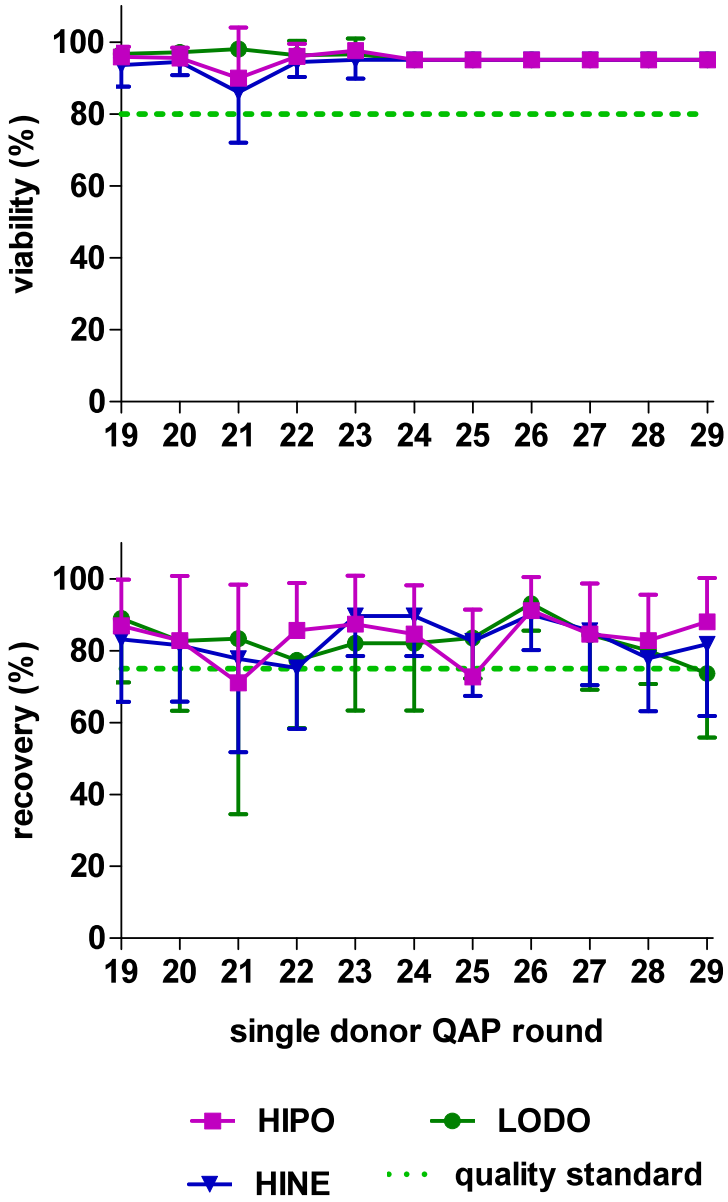

Figure 2. Cumulative trend in viability and post thaw recovery compared with the 10 previous QA rounds.
Mean and standard deviation; recovery results >100% were rounded down to a maximum recovery of 100%.

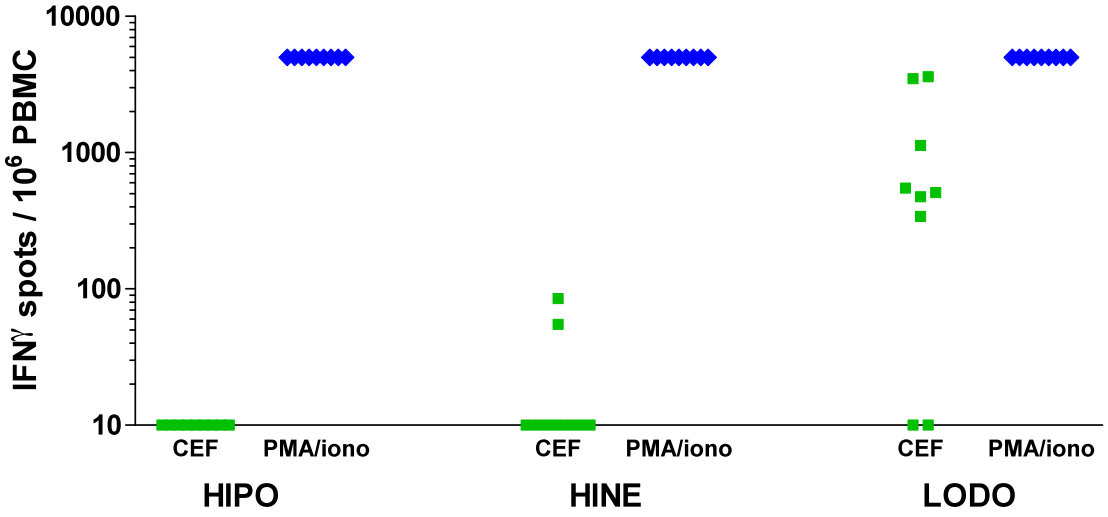

Figure 3. PBMC function results determined by IFN-γ ELISPOT. Antigen-specific responses were determined by stimulation and overnight culture with the CEF peptide pool, and maximal cytokine release with PMA + ionomycin.

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
	27th round	28th round	29th round	(passed 2 of 3 QAP rounds)
B	yes	no	yes	**Certified**
E	yes	yes	yes	**Certified**
F	yes	yes	no	**Certified**
J	yes	yes	no	**Certified**
K	yes	yes	yes	**Certified**
M	yes	yes	yes	**Certified**
O	yes	yes	yes	**Certified**
P	yes	yes	yes	**Certified**
R	yes	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.