Report: 34th IVRN PBMC cryopreservation QA round, Nov 2019

Executive Summary

The 34th IVRN QA exercise took place on 6th Nov 2019, and assessment of returned PBMC specimens was completed in Dec 2019. The primary outcomes of this QA round are:

- Efficient PBMC fractionation;
- Continued improvements in post-thaw recovery;
- Good PBMC function despite low response from the HIV-positive donor;
- All of 11 participating laboratories passed this QA round, and are designated certified for PBMC cryopreservation.

PBMC fractionation recovery

Fractionation recovery was calculated from the full blood differential counts provided from participating labs (Table 1). The mean fractionation recovery was 49%, which is the expected level of recovery from careful Ficoll centrifugation. The minimum accepted fractionation recovery from the local donor specimen was >1 x 10^6 PBMC per 1ml blood if a FBC was not performed. Fractionation recovery was uniformly high, with the exception of lab B (Table 2). The lymphocyte count was used as the total PBMC number, however inclusion of monocytes would not have increased recovery to an acceptable level. The 120% post-thaw recovery suggested counts were underestimated, perhaps due to a dilution error.

Table 1. Total PBMC in 30ml whole blood samples for 34th QA round, reported from each lab on the day of 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 P	52.17	61.38	Coulter Act Diff
lab T	NA	53.79	Coulter DxH520
Lab U			DellDyn Sapphire
24hr bloods (average)	54.3 x10^6	54.9 x10^6	

Post-thaw 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. Small cell clumps formed after cell sedimentation in several thawed specimens, and was likely the result of neutrophils present after fractionation. Since these small clumps were ignored if present on the haemocytometer, the resulting viability was uniformly high (Table 2); the lowest viability was 88% as confirmed by manual counting.

Post-thaw PBMC recovery was uniformly high, <75% in only two PBMC specimens, associated with an unlikely high fractionation recovery. Recovery in four specimens was >125%. An inverse association between apparently low fractionation recovery and excess post-thaw recovery is shown in Figure 1. Note how the HINE specimen from Lab F had the highest fractionation recovery but

lowest thawed recovery, but had an absolute recovery within the group average range (Fig 1C). Lab B specimens showed both low fractionation and absolute recovery, suggesting inadequate fractionation skills if not for the local donor specimen (the local donor specimen can play a crucial role in the QA process). The mean absolute recovery of all PBMC specimens from this round was 47%, suggesting overall proficiency in extraction and cryopreservation of viable PBMC from whole blood samples. The cumulative trend in viability and post-thaw recovery over the past 10 QAP rounds is shown in Figure 2.

Functional analysis

PBMC function was determined by IFNγ ELISPOT assay, measuring the response to the CEF peptide pool (epitopes from CMV, EBV and Influenza), and maximal stimulation from PMA and ionomycin (Figure 3). We used blood from the same HIV-pos donor as from previous QA rounds, hence responses to the CEF peptide pool were low again. The HIV-neg donor had a strong response, while individual local donors varied from undetectable to strong. All PBMC samples showed maximal stimulation in the presence of PMA and ionomycin (>5000 spots/million PBMC). Specimens from Lab F continue to show a high frequency of responder cells in control wells. This may be caused by to some stimulus during PBMC preparation, possibly from the FBS used, or the medium may have low level LSP contamination. Unlike the 33rd QA round, the same HIV-pos donor PBMC did not exhibit high background in the current round. Since the natural level of background activation in local donor specimens is not known, ELISPOT results from Lab F's local donor were accepted as a pass, although also being high. An investigation into the cause of this high background at Lab F is warranted.

Overall conclusions on performance in the 34th QA round

All labs achieved uniformly high viability results, and good post thaw recovery. 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.

34th IVRN QAP report was produced by Dr Wayne Dyer, on behalf of the IVRN Executive.

Table 2. 34th 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	blood PBMC	[1] fractionation recovery (%)	thawed count (10^6)	[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/recovery	[4] Adequate response in function assays	[5] overall result
B	HIV-pos	20/05/2019	30	7	1	7	47.08	14.9	7.335	104.8	15.6	>95	3	0	>5000	no	yes	yes	
	HIV neg	20/05/2019	30	9	1	9	40.67	22.1	10.824	120.3	26.6	>95	2	1010	>5000	no	yes	yes	pass
	local donor	21/05/2019	16.5	7.15	2	14.3	44.86	31.9	5.928	82.9	26.4	>95	1	1210	>5000	yes	yes	yes	
E	HIV-pos	20/05/2019	30	7.1	2	14.2	47.08	30.2	9.500	133.8	40.4	>95	5	0	>5000	yes	high	yes	
	HIV neg	20/05/2019	30	8.6	2	17.2	40.67	42.3	8.279	96.3	40.7	>95	3	1760	>5000	yes	yes	yes	pass
	local donor	21/05/2019	27	9.7	2	19.4	37.8	51.3	7.816	80.6	41.4	>95	4	1220	>5000	yes	yes	yes	
F	HIV-pos	20/05/2019	30	10.5	2	21	47.08	44.6	9.054	86.2	38.5	>95	64	0	>5000	yes	yes	high background	
	HIV neg	20/05/2019	30	9.6	3	28.8	40.67	70.8	6.908	72.0	51.0	>95	125	800	>5000	yes	no	high background	pass
	local donor	21/05/2019	27	12	3	36		OK	10.769	89.7	NA	>95	38	1010	>5000	yes	yes	yes	
J	HIV-pos	20/05/2019	30	5	4.7	23.5	47.08	49.9	6.279	125.6	62.7	>95	4	0	>5000	yes	yes	yes	
	HIV neg	20/05/2019	30	5	4.4	22	40.67	54.1	4.800	96.0	51.9	>95	6	1240	>5000	yes	yes	yes	pass
	local donor	21/05/2019	20	5	5.5	27.5	30.19	91.1	3.500	70.0	63.8	>95	0	170	>5000	yes	no	yes	
K	HIV-pos	20/05/2019	30	6.6	3	19.8	47.08	42.1	5.165	78.3	32.9	>95	13	0	>5000	yes	yes	yes	
	HIV neg	20/05/2019	30	7.6	3	22.8	40.67	56.1	8.902	117.1	65.7	88	18	900	>5000	yes	yes	yes	pass
	local donor	21/05/2019	30	9.37	4	37.48		OK	9.890	105.5	NA	>95	1	0	>5000	yes	yes	yes	
M	HIV-pos	20/05/2019	30	8.88	2	17.76	47.08	37.7	12.225	137.7	51.9	>95	4	0	>5000	yes	high	yes	
	HIV neg	20/05/2019	30	10.23	2	20.46	40.67	50.3	11.029	107.8	54.2	>95	3	950	>5000	yes	yes	yes	pass
	local donor	21/05/2019	60	12.35	6	74.1	167.03	44.4	11.688	94.6	42.0	>95	4	660	>5000	yes	yes	yes	
O	HIV-pos	20/05/2019	30	5.88	4	23.52	47.08	50.0	6.279	106.8	53.3	>95	9	0	>5000	yes	yes	yes	
	HIV neg	20/05/2019	30	5.37	4	21.48	40.67	52.8	4.500	83.8	44.3	>95	8	1540	>5000	yes	yes	yes	pass
	local donor	21/05/2019	12	5.92	6	35.52		OK	6.797	114.8	NA	>95	1	1160	>5000	yes	yes	yes	
P	HIV-pos	20/05/2019	30	9.65	2	19.3	47.08	41.0	13.514	140.0	57.4	>95	6	0	>5000	yes	high	yes	
	HIV neg	20/05/2019	30	11.25	2	22.5	40.67	55.3	10.615	94.4	52.2	>95	2	1780	>5000	yes	yes	yes	pass
	local donor	21/05/2019	30	12.9	2	25.8	44.21	58.4	9.730	75.4	44.0	>95	1	420	>5000	yes	yes	yes	
R	HIV-pos	20/05/2019	30	5.7	3	17.1	47.08	36.3	4.500	78.9	28.7	>95	5	0	>5000	yes	yes	yes	
	HIV neg	20/05/2019	30	5.8	3	17.4	40.67	42.8	4.870	84.0	35.9	>95	3	1810	>5000	yes	yes	yes	pass
	local donor	21/05/2019	17	6.1	4	24.4	46.22	52.8	4.890	80.2	42.3	>95	1	1630	>5000	yes	yes	yes	
T	HIV neg	20/05/2019	30	6.125	4	24.5	40.67	60.2	5.269	86.0	51.8	>95	21	1550	>5000	yes	yes	yes	pass
	local donor	21/05/2019	57	6.16	10	61.6	87.78	70.2	4.915	79.8	56.0	>95	1	0	>5000	yes	yes	yes	
U	HIV-pos	20/05/2019	30	6.85	3	20.55	47.08	43.6	6.762	98.7	43.1	>95	10	0	>5000	yes	yes	yes	
	HIV neg	20/05/2019	30	7.74	3	23.22	40.67	57.1	7.305	94.4	53.9	>95	3	1580	>5000	yes	yes	yes	pass
	local donor	21/05/2019	30	8.32	4	33.28	55.39	60.1	14.007	168.4	101.2	>95	1	600	>5000	yes	high	yes	

Notes: **(1) Assessment criteria 1:** fractionation recovery >30% of available PBMC = 47.08 & 40.67 million PBMC/30ml blood; HIPO & HINE, respectively.

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 visualised 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 haematology cell counter.

(4) Assessment criteria 4: ELISPOT results: PMA/Ionomycin: >5000/10^6 PBMC (all samples); CEF (mean - 2SD) = 0 & >472 x 10^6 PBMC (HIV+ & neg); control spots (mean +2SD) <49 & <87 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 — Results that failed the assessment criteria.

Orange — Actual PBMC count was higher than stated, resulting inhigh fractionation recovery but low post thaw recovery.

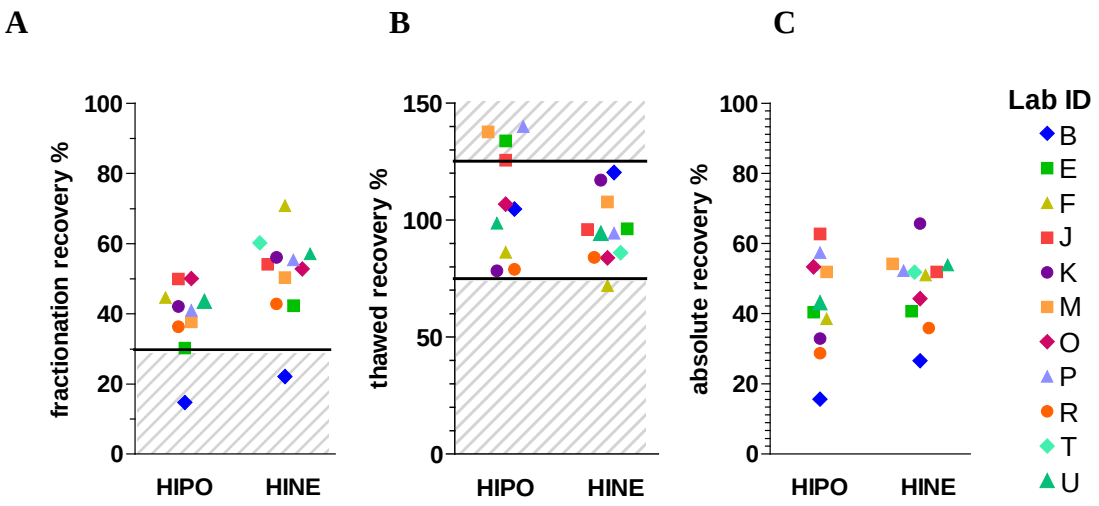

Figure 1. Comparison of relative vs. absolute recovery of PBMC showing (A) post fractionation recovery relative to laboratory cell count; (B) thawed PBMC recovery relative to laboratory cell count, and (C) absolute recovery of PBMC (total thawed PBMC x number of vials) expressed as the % of the mean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

Figure 2. Viability and post thaw recovery compared with the 10 previous QA rounds.
Mean and standard deviation; the maximum post-thaw recovery was defined as 100%.

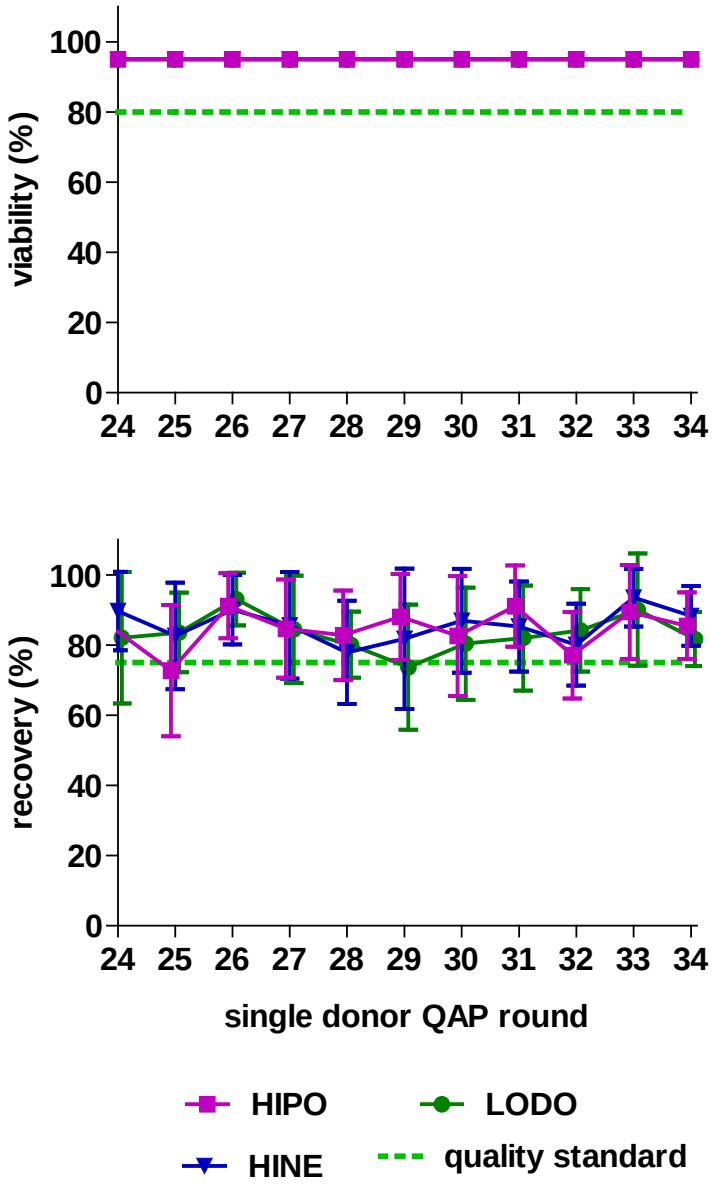

Figure 3. PBMC function results determined by IFN-γ ELISPOT. Antigen-specific responses were determined by stimulation and overnight culture with the CEF peptide pool, and maximal cytokine release with PMA + ionomycin.

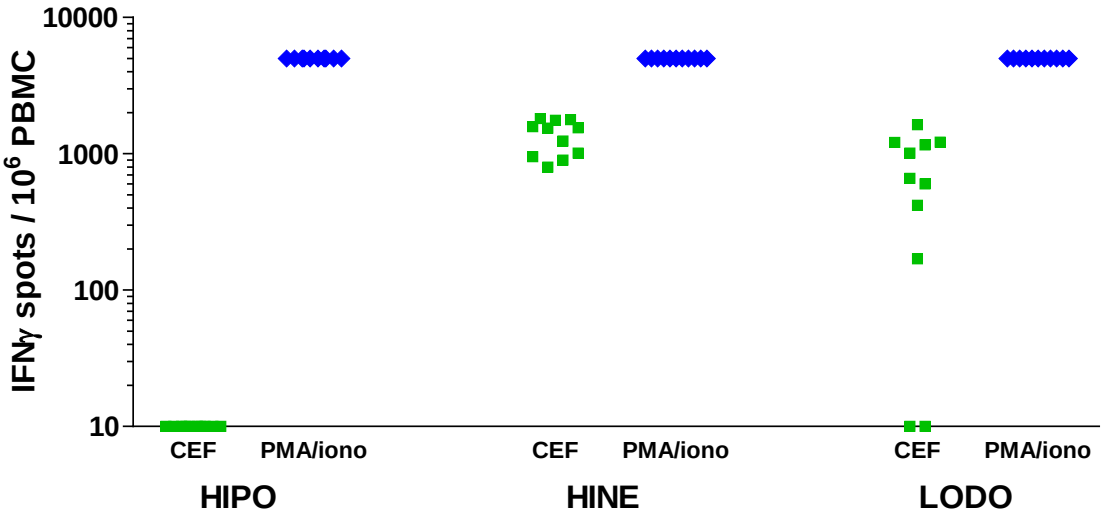

Table 3. Current certification status of Tier 1 labs.

lab code	Adequately performance over the previous QAP rounds? (all 4 quality standards met in at least one PBMC specimen)			current status
	32nd round	33rd round	34th round	(passed 2 of 3 QAP rounds)
B	pass	pass	pass	**Certified**
E	pass	pass	pass	**Certified**
F	pass	pass	pass	**Certified**
J	pass	fail	pass	**Certified**
K	fail	pass	pass	**Certified**
M	fail	NA	pass	**Certified**
O	pass	pass	pass	**Certified**
P	pass	pass	pass	**Certified**
R	pass	pass	pass	**Certified**
T	pass	NA	pass	**Certified**
U		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 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.