Introduction to the IQA Cryopreservation PT Program

The purpose of the Immunology Quality Assessment (IQA) Cryopreservation Proficiency Testing (PT) Program is to provide a resource to evaluate and enhance the ability of U.S. and non-U.S. laboratories participating in National Institute of Allergy and Infectious Diseases (NIAID) - Division of AIDS (DAIDS) funded clinical study protocols. The leadership of the AIDS Clinical Trials Group (ACTG) and the International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) requires that Clinical Trial Units participate in a quarterly proficiency testing program to evaluate the ability of labs to reliably cryopreserve viable peripheral blood mononuclear cell (PBMC) samples. The IQA Cryopreservation PT Program measures the viability and viable recovery of PBMC samples processed at network laboratories on a quarterly basis to ensure PBMC sample integrity in support of NIAID-DAIDS network studies.

- Viability is a measurement of the portion of PBMC cells in the sample that are alive. Viable cells are required to perform successful functional analyses.

- Viable recovery is a comparison between the number of viable PBMC cells cryopreserved at the lab and the number of viable PBMC cells retrieved after thawing. A low viable recovery indicates that there are not enough cells to complete the required analyses. Inflated viable recovery indicates that cells are not being distributed efficiently for network protocols.

IQA Cryopreservation PT Program Enrollment

- A newly enrolling network laboratory is required to complete a prequalification procedure prior to actively participating in the IQA Cryopreservation PT Program.
- The prequalification process is equivalent to two quarters, per the quarterly PT requirements, this requires a shipment of 8 PBMC aliquots (2 PBMC aliquots from 4 donors) to the IQA.
- PBMC samples collected and frozen from 2 of the 4 donors should occur on different days.
- The prequalification samples may be submitted at any time outside of the regularly scheduled quarter.
- The laboratory must notify the IQA Cryopreservation PT Program staff in advance for the samples to be evaluated out of the normally scheduled PT quarter.
- The laboratory must obtain an Approved or Provisionally Approved status, refer to performance evaluation scoring method, to participate in a network protocol that require the collection of viable cryopreserved PBMC samples.
- After the successful completion of the prequalification process, required participation will begin in the next regularly scheduled IQA Cryopreservation PT quarter.
- If acceptable results are not obtained, the lab must repeat the prequalification process per the corresponding networks discretion.

Quarterly PT Requirements

Each laboratory is ***required*** to submit 2 aliquots from 2 donors with each quarter to maintain eligibility to cryopreserve PBMC samples for network protocols. Additional samples should not be submitted to the IQA, labs are encouraged to keep the extra aliquots on site in case there is a need for an in lab investigation and/or courier delays. Labs are provided detailed instructions prior to the start of each quarter on the following requirements:

- Collection of blood from 2 donors (Either HIV +/-).
- Isolation and cryopreservation of PBMC samples per the Cross Network PBMC Processing SOP https://www.hanc.info/resources/sops-guidelines-resources/laboratory/cross-network-pbmc-processing-sop.html.
- If applicable, labs processing for more than one network protocol are encouraged to alternate PBMC cryopreservation procedures based on the network SOPs (i.e. HVTN).

- Labs must submit 2 aliquots from each of the 2 donors for a total of 4 aliquots each quarter.
- Each aliquot should contain a viable cell concentration of ~5 x 10^6 cells per cryovial, but concentrations as low as 3 x 10^6 cells per cryovial are accepted.
- PBMC samples must be stored frozen on site for at a minimum of 1 week, and a maximum of 5 weeks before shipping to the IQA.
- Labs must complete the LDMS IQA Cryopreservation and Viability Data Entry and provide the IQA with a shipping file for import.
- Labs must properly pack an expedited shipment to the IQA laboratory before the final quarterly shipping deadline.

Performance Evaluation Scoring Method

A performance evaluation scoring method is used to evaluate lab performance and to determine whether the lab is qualified to cryopreserve PBMC samples for network protocols**.** The percent viability and viable recovery is assessed within each round of testing to determine the laboratory status.
PBMC aliquots from each donor are analyzed by the IQA, per the IQA PBMC Thawing SOP available on the IQA website https://iqa.center.duke.edu/resources/cryopreservation/processing. The number of aliquots analyzed by the IQA is dependent upon the initial aliquot's percent viability and viable recovery immediately after thawing. The percent viability and viable recovery are each issued a score, see Figure 1.0, Percent Viability Scoring System and Figure 2.0, Percent Viable Recovery Scoring System. The quarterly IQA Cryopreservation PT report includes the percent viability and viable recovery for a total of 2 aliquots, one aliquot selected from each of the 2 donors based on the best possible score. The 2 scores from both percent viability and viable recovery are combined to determine the status for each parameter, see Figure 3.0, Determining the Status for Percent Viability and Viable Recovery. The 2 statuses, percent viability and viable recovery, are further combined to determine the overall status, see Figure 4.0 Determining the Overall Status for Network Protocol Participation. The overall status determines eligibility of the lab to continue to process PBMC samples for network protocols. A lab must maintain an overall status of an Approved and/or at least a Provisionally Approved to continue eligibility for active protocols that cryopreserve viable PBMC samples, see Figure 5.0, Overall Status Specifications. Language is included in the quarterly IQA Cryopreservation PT report to prompt the lab to complete the required corrective actions in a timely manner:

1. IQA Investigation Report (IR) Form:

    - If a lab receives a viability and/or viable recovery score of 0 or 1 on any one sample, it is required to complete an IQA Investigation Report within 5 working days.
        - The IQA will communicate directly with the laboratory staff to identify and resolve possible underlying challenges (i.e., staff training, processing difficulties, counting errors).
        - The IQA will review the IR form for acceptability and provide the finalized IR to the lab and the corresponding networks.

2. Required to resubmit the equivalent of one quarter (Labs must submit 2 aliquots from each of the 2 donors for a total of 4 aliquots) of samples within 4 weeks that result in an Approved status:

    - If a lab receives an On Probation status for a given quarter.
    - If a lab receives a Provisionally Approved status in 2 consecutive quarters.

        - Labs will be placed On Hold and will not be able to process PBMCs for protocols that require viable PBMC samples until an Approved status is achieved for protocols where viable PBMC are used for assessing **S**afety, **P**articipant management, **E**ligibility, or a primary **E**ndpoint, or Diagnosis (SPEED criteria). The Laboratory Center in conjunction with DCLOT will decide if viable PBMC are used for this purpose in a particular protocol.
        - An IQA-certified back-up laboratory must be identified to process the primary laboratory's protocol PBMC samples until the primary lab improves its Probation Status to Approve status for all protocols where viable PBMC are used for testing that meets SPEED criteria. Please contact the ACTG/IMPAACT and the IQA for discussion about

back-up options.

- For labs in areas where a backup lab is not available, continuation of PBMC processing will be left at the discretion of network Laboratory Center.

Figure 1.0: Percent Viability Scoring System

Percent Viability	Score	Interpretation
≥80% to 100%	2	This is the optimal viability percentage to conduct on-site and downstream network assays.
≥65% to 79%	1	This is less than an optimal viability percentage, and there is reason to be concerned about the ability of the lab to process network study samples. The lab is ***required*** to complete an IR form and work with the IQA to improve PBMC processing performance.
<65%	0	This is an unacceptable score, network protocols using viable PBMC samples would not be able to conduct the required functional assays. The lab is required to complete an IR form and work with the IQA to improve PBMC processing performance.

Figure 2.0: Percent Viable Recovery Scoring System

Percent Viable Recovery	Score	Interpretation
≥70% to 120%	2	This is the optimal viable recovery percentage to conduct on-site and downstream network assays.
≥50% to >69%	1	This is less than an optimal viable recovery percentage, and there is reason to be concerned about the ability of the lab to collect enough PBMC in each aliquot required to complete network studies. The lab is ***required*** to complete an IR form and work with the IQA to improve PBMC processing performance.
≥120% to 150%	1	This is less than an optimal viable recovery percentage, and there is reason to be concerned about the ability of the lab to collect enough PBMC in each aliquot required to complete network studies. The lab is ***required*** to complete an IR form and work with the IQA to improve PBMC processing performance.
<50%	0	This is an unacceptable score, network protocols using viable PBMC samples would not be able to conduct the required functional assays. The lab is required to complete an IR form and work with the IQA to improve PBMC processing performance.
>150%	0	This is an unacceptable score, network protocols using viable PBMC samples would not be able to conduct the required functional assays. The lab is required to complete an IR form and work with the IQA to improve PBMC processing performance.

Figure 3.0: Determining the Status for Percent Viability and Viable Recovery

- The viability scores for each sample are combined to yield the viability percentage status.

Combined Viability Score	Viability % Status
3-4	A (Approved)
2	PA (Provisional Approved)
0-1	OP (On Probation)

- The viable recovery scores for each sample are combined to yield the viable recovery status.

Combined Viability Score	Viable Recovery %Status
3-4	A (Approved)
2	PA (Provisional Approved)
0-1	OP (On Probation)

Figure 4.0: Determining the Overall Status for Network Protocol Participation

Viability % Status	Viable Recovery % Status	Overall Status
A	A	A (Approved)
A	PA	PA (Provisionally Approved)
PA	A	PA (Provisionally Approved)
PA	PA	PA (Provisionally Approved)
A	OP	OP (On Probation)
OP	A	OP (On Probation)
PA	OP	OP (On Probation)
OP	PA	OP (On Probation)
OP	OP	OP (On Probation)

Figure 5.0 Overall Status Specifications:

Eligibility/Corrective action Requirements	**A (Approved)**	**PA (Provisionally Approved)**	**PA (Provisionally Approved) in 2 consecutive quarters**	**OP (On Probation)**
Eligible to continue to perform PBMC cryopreservation for ACTG/IMPAACT protocols?	Yes	Yes	No*	No*
Eligible to start PBMC Cryopreservation for new ACTG/IMPAACT protocols?	Yes	Yes	No	No
Eligible to serve as an IQA- certified back-up laboratory?	Yes	No	No	No
Required to complete an IQA Investigational Report form for any sample with a score of < 1, and work with the IQA to improve performance?	Yes	Yes	Yes	Yes
Required to resubmit the equivalent of one quarter of samples within 4 weeks that result in an overall (A) Approved status?	No	No	Yes	Yes
Required to use a backup lab, where applicable?	No	No	Yes*	Yes*
*Labs will be placed ON HOLD and required to use an approved back-up laboratory until the lab improves probation status for all protocols where viable PBMC meet SPEED criteria. For labs in areas where a backup lab is not available, continuation of PBMC processing will be left at the discretion of the Laboratory Center.				