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 Advancing Clinical Therapeutics Globally for HIV/AIDS and Other Infections (ACTG) and the International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) requires that all technicians who process PBMCs for their clinical trials participate in a quarterly proficiency testing program to evaluate the ability of staff 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 laboratories and shipped to the IQA on a quarterly basis to ensure PBMC sample integrity in support of NIAID-DAIDS 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 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 study protocols.

IQA Cryopreservation PT Program Enrollment

Newly enrolling labs must have each processing technician submit 2 PBMC aliquots from 1 donor to the IQA. A maximum of 4 technicians/laboratory are allowed to be certified for PBMC processing. PBMC samples collected and frozen from 2 of the 4 donors should occur on different days. Samples may be submitted at any time. Each aliquot should contain a viable cell concentration of ~5 x 10^6 cells per cryovials, but concentrations as low as 3 x 10^6 cells per cryovial are acceptable. PBMC samples must be stored at -65°C to -95°C on site for a minimum of 1 week and a maximum of 5 weeks before shipping on dry ice to the IQA. Labs must notify the IQA Cryopreservation PT Program staff prior to shipment. Once satisfactory performance is achieved, the lab and the corresponding processing technicians will be enrolled to participate in the quarterly rounds of PT, which will begin with the next scheduled IQA Cryopreservation PT quarter. Any technician who receives an Unsatisfactory score must resubmit samples for evaluation and receive a satisfactory score prior to proceeding with processing PBMCs for network protocols. Technicians from each lab must be participating in quarterly rounds of PT and maintain a satisfactory score **in each participating round** in order to be able to collect and store PBMCs for protocols.

Enrollment in the program does not equate to approval to process PBMCs for protocols. Approval to process PBMC for protocols is granted by the Networks on a protocol by protocol basis. Approval is not granted by the IQA.

Refer to the following links for PBMC processing resources:

- https://www.hanc.info/resources/sops-guidelines-resources/laboratory/actg-impaact-laboratory-resources.html
- https://www.hanc.info/resources/sops-guidelines-resources/laboratory/cross-network-pbmc-processing-sop.html

Quarterly PT Requirements

To prevent disrupting the normal flow of protocols, each laboratory should have several qualified staff members capable of processing PBMCs. Once a laboratory has completed the prequalification process, they will proceed with quarterly submissions. A maximum of 2 processors are allowed to submit quarterly. These submissions will consist of 2 aliquots from a single donor.

Laboratories with only 1 staff member capable of PBMC processing will submit 2 aliquots from a single donor quarterly. Laboratories with more than 2 staff members capable of PBMC processing are expected to maintain a regular rotation of up to 4 processors, with 2 sets of 2 technicians submitting samples in alternating quarters. This ensures that the submissions are still within the 4 aliquot limit and consistent participation. It is the laboratory's responsibility to maintain and monitor the certification and participation status of each processor. If a technician misses 2 consecutive rounds of participation, they will be ineligible to process PBMCs until they submit a passing sample.

The IQA recommends keeping additional aliquots from the submissions on site in the event that they are needed for an in-lab further 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 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
- Labs must submit 2 aliquots/donor per 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 acceptable.
- PBMC samples must be stored at -65°C to -95°C on site for 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, the IQA Web-Based System Site Data submission entry, and provide the IQA with a shipping file for import via email.
- Labs must properly pack an expedited shipment to the IQA laboratory before the final quarterly shipping deadline.
- Shipment must not be exposed to gamma radiation or X-rays.

Performance Evaluation Scoring Method

A performance evaluation scoring method is used to evaluate lab performance. The percent viability and viable recovery are assessed within each round of testing to determine the lab performance grading. PBMC aliquots from each donor are analyzed by the IQA, per the IQA PBMC Thawing SOP, available on the IQA website (https://dhvi.duke.edu/programs-and-centers/immunology-virology-quality-assessment-center/research-programs/immunology-5). The number of aliquots analyzed 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 each sample. Each sample will receive individual scores for both percent viability and viable recovery. This score will determine the performance grading for each parameter (see ***Figure 3.0, Determining Performance Grading for Percent Viability and Viable Recovery***.)

The IQA will issue a Potential Issue Alert (PIA) email to labs and the applicable technician(s) whose result report indicates that there may be a potential issue that could cause a future Unsatisfactory performance status that may need further investigation. This informal notice does not require a response from the lab.

Technicians that receive an Unsatisfactory performance grading will be required to complete the following 2 items:

1. IQA Investigation Report (IR) Form:
    - The IQA IR form must be completed within 5 working days of receipt of the result report.
        - The technician may request a troubleshooting meeting with the IQA to identify and resolve possible underlying challenges (i.e. staff training, processing difficulties, counting errors, etc.)
        - The IQA will review the IR form for acceptability and provide the finalized IR to the lab, technician(s), and the corresponding networks.
2. Resubmission samples
    - The technician(s) must resubmit 2 aliquots from 2 separate samples to the IQA within 4 weeks of receiving the result report.
    - During this time, the lab and the technician(s) will need to reach out to the network Laboratory Center to provide a plan for recertification and/or continued PBMC processing.
    - At the end of the quarter, the IQA provides the Laboratory Center a list of laboratories and technicians that have received an **Unsatisfactory** performance grading.

The technician and/or laboratory that has received an Unsatisfactory score will not be able to process PBMCs for network protocols until they are again in good standing. It is the responsibility of the Laboratory Director to reach out to the Laboratory Center of relevant Network(s) any time they receive an Unsatisfactory Score to provide a plan for recertification, and to provide the Laboratory Center with a listing of protocols potentially impacted.

The Processing Lab Laboratory Director should work with the Data Management Center to determine the number of participant visits that might be impacted during the time frame required for resubmitting samples to the IQA. The Laboratory Center will work with the processing laboratory to help to identify potential back up laboratories that could be used for processing PBMC during this time if needed. **Note**: **if there is >1 technician certified for a laboratory, any technician who has received a satisfactory score on their last submission and who has participated as part of their regularly scheduled rotation, is allowed to continue processing PBMCs.**

If a laboratory does not have any qualified technicians, then the laboratory cannot collect protocol PBMCs during the recertification process. Protocols that include language indicating that collection of PBMCs is only required by laboratories in good standing with the IQA can opt not to collect PBMCs during this time period if a suitable back up laboratory cannot be identified. It is the Laboratory Director and Site PI's responsibility to notify protocol teams and the Laboratory Center when they are unable to collect PBMCs.

Figure 1.0: Percent Viability Scoring System

Percent Viability	Score	Interpretation
≥80% to 100%	2	This is the optimal viability percentage.
≥70 to 79.9%	1	This is less than optimal viability percentage.
<70%	0	This is an unacceptable score.

Figure 2.0: Percent Viable Recovery Scoring System

Percent Viable Recovery	Score	Interpretation
≥80% to 120%	2	This is the optimal viable recovery percentage.
≥70% to 79.9%	1	This is less than an optimal viable recovery percentage.
>120% to 130%	1	This is less than an optimal viable recovery percentage.
<70%	0	This is an unacceptable score.
>130%	0	This is an unacceptable score.

Figure 3.0: Determining Performance Grading for Percent Viability and Viable Recovery

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

Viability Score	Viability Status
1-2	Satisfactory
0	Unsatisfactory

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

Viable Recovery Score	Viable Recovery Status
1-2	Satisfactory
0	Unsatisfactory

Figure 4.0: Determining the Performance Grading per Technician

Viability Status	Viable Recovery Status	Overall Status
Satisfactory	Satisfactory	Satisfactory
Satisfactory	Unsatisfactory	Unsatisfactory
Unsatisfactory	Satisfactory	Unsatisfactory
Unsatisfactory	Unsatisfactory	Unsatisfactory