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 laboratories 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 are required to submit 8 PBMC aliquots, composed of 2 PBMC aliquots from 4 separate donors, to the IQA. 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 accepted. PBMC samples must be stored at -65˚C to -80˚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 will participate in the quarterly rounds of PT, which will begin with the next scheduled IQA Cryopreservation PT quarter. Enrollment in the program does not indicate approval to process PBMCs for protocols.

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

Each lab is ***required*** to submit 2 aliquots from 2 separate donors, for a total of 4 vials, with each quarter to obtain/maintain eligibility to cryopreserve PBMC samples for network protocols. The IQA recommends keeping additional aliquots from the submissions on site in the event that they are needed 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
- 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 at -65˚C to -80˚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 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 a total of 2 aliquots, composed of 1 aliquot from each sample selected based on the best possible score. The 2 scores from both percent viability and viable recovery are combined to determine the performance grading for each parameter (see Figure 3.0, Determining Performance Grading for Percent Viability and Viable Recovery.) Percent viability grading and viable recovery grading are combined to determine the overall performance grading (see Figure 4.0, Determining Overall Performance Grading.)

The IQA will issue a Potential Issue Alert (PIA) email to labs 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.

Labs 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 IQA will communicate directly with the lab staff 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 and the corresponding networks.
2. Resubmission samples
    - Labs must resubmit 2 sets of quarterly samples to the IQA within 4 weeks of receiving the result report.
    - During this time, the lab will need to reach out to the network Laboratory Center in regards to continued PBMC processing.
    - At the end of the quarter, the IQA provides the Laboratory Center a list of laboratories that have received an **Unsatisfactory** performance grading.

Figure 1.0: Percent Viability Scoring System

Percent Viability	Score	Interpretation
≥80% to 100%	2	This is the optimal viability percentage.
≥65 to 79.9%	1	This is less than optimal viability percentage.
<65%	0	This is an unacceptable score.

Figure 2.0: Percent Viable Recovery Scoring System

Percent Viable Recovery	Score	Interpretation
≥70% to 120%	2	This is the optimal viable recovery percentage.
≥50% to 69.9%	1	This is less than an optimal viable recovery percentage.
>120% to 150%	1	This is less than an optimal viable recovery percentage.
<50%	0	This is an unacceptable score.
>150%	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.

Combined Viability Score	Viability Status
2-4	Satisfactory
0-1	Unsatisfactory

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

Combined Viable Recovery Score	Viable Recovery Status
2-4	Satisfactory
0-1	Unsatisfactory

Figure 4.0: Determining the Overall Performance Grading

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