DAIDS IQA PBMC Cryopreservation Assessment Program Introduction

The purpose of the Division of AIDS (DAIDS) Immunology Quality Assessment (IQA) Peripheral Blood Mononuclear Cell (PBMC) Cryopreservation (Cryo) Assessment 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 and cryopreserve viable PBMCs for their clinical trials participate in a quarterly assessment program to evaluate the ability of staff to reliably cryopreserve viable PBMC samples. The DAIDS IQA PBMC Cryopreservation Assessment 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.

DAIDS IQA PBMC Cryopreservation Assessment Program Enrollment

Newly Enrolling Laboratories

- A newly enrolling laboratory will be required to identify their laboratory's PBMC sample processors for the program. A maximum of 4 technicians within a laboratory is allowed to be certified for PBMC processing. Each processor must submit 2 PBMC aliquots from 1 donor to the IQA for evaluation. PBMC samples collected and frozen from 2 of the 4 donors should occur on different days. Newly enrolling laboratories may submit samples at any time.

Adjustment in PBMC Sample Processor List

- Laboratories who are already enrolled in the program must notify the IQA about their updated PBMC processor list, along with a request to complete the prequalification for the PBMC Sample Processor that is added to the rotation. The processor must submit 2 PBMC aliquots from 1 donor to the IQA for evaluation.

Each aliquot should contain a viable cell concentration of 3-5 x 10^6 cell per cryovials. 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 (*Note*: the cryopreserved PBMCs must be placed in liquid nitrogen vapor phase within 5 weeks of collection). Laboratories must notify the DAIDS IQA PBMC Cryopreservation Assessment Program staff prior to shipment. Once satisfactory performance is achieved, the certified processing technicians will be enrolled to participate in the

quarterly rounds of Assessment, which will begin with the next scheduled DAIDS IQA PBMC Cryopreservation Assessment quarter. Any technician who receives an unsatisfactory score must resubmit samples for evaluate and receive a satisfactory score prior to processing PBMCs for network protocols. Technicians from participating laboratories should participate in or rotate across quarterly rounds of Assessment and maintain a satisfactory score to be eligible to process and cryopreserve PBMCs for ACTG and IMPAACT protocols; up to two technicians may submit PBMC samples for each quarter.

Enrollment in the program does not equate to approval to process PBMCs for protocols. Approval to process PBMC for protocols is granted by the Clinical Trial Network on a protocol-by-protocol basis.

Refer to the following links for PBMC processing resources:

- Cross-Network PBMC Processing SOP: https://www.hanc.info/resources/sops-guidelines-resources/laboratory/cross-network-pbmc-processing-sop.html
- For IMPAACT PBMC Processing SOP: https://actg-impaact-lc.org/resources/documents/
- For Fetal Bovine Serum (FBS) Ordering Information: https://actg-impaact-lc.org/resources/documents/
- Cross-Network PBMC Processing Worksheet Examples: https://actg-impaact-lc.org/resources/documents/

DAIDS IQA PBMC Cryopreservation Assessment Program Quarterly Requirements

Laboratories may have up to 4 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 is allowed to submit PBMC samples on a quarterly basis. 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 laboratory technicians maintain consistent participation. It is the laboratory's responsibility to 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.

If there is sufficient cells, the IQA requires laboratories to keep the additional aliquots from the submissions on site so they may be used for further intra-laboratory investigation (as needed) or for repeat shipping if the samples are not received by the IQA in good condition due to courier delays. Laboratories are provided detailed instructions prior to the start of each quarter on the following requirements:

- The donor requirements (either HIV +/-) for blood Collection
- Reference to the Cross Network PBMC Processing SOP https://www.hanc.info/resources/sops-guidelines-resources/laboratory/cross-network-pbmc- processing-sop.html
- Expected sample submission requirements (e.g., Laboratories must submit 2

aliquots/donor per quarter).

- Samples concentration requirements for submission (e.g., Each aliquot should contain a viable cell concentration of 3-5 x 10^6 cells per cryovial).
- Storage requirements for PBMC samples (e.g., PBMC 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).
- Requirements for Data entry and email notifications (e.g., 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 LDMS Batch shipping file, LDMS Shipping Manifest, and Tracking Information via email).
- Shipping start dates and shipping deadlines for each quarterly shipment.
- Reminders that shipment must not be exposed to gamma radiation or X-rays.

DAIDS IQA PBMC Cryopreservation Assessment Performance Evaluation Scoring Method

A performance evaluation scoring method is used to evaluate PBMC processing performance. The percent viability and viable recovery are assessed within each round of testing to determine the PBMC processing performance rating.

PBMC aliquots from each donor are analyzed by the IQA, per the *IQA PBMC Thawing SOP*, available on the IQA website under IQA Resources > Cryopreservation (https://dhvi.duke.edu/programs-and-centers/immunology-virology-quality-assessment-center/research-programs/immunology-4). 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 *DAIDS IQA PBMC Cryopreservation Assessment Report* includes the percent viability and viable recovery score for each sample. Each sample will receive individual scores for both percent viability and viable recovery. This score will determine the performance rating for each parameter (**see *Figure 3.0, Determining Performance Grading for Percent Viability and Viable Recovery***.)

For technicians that received a less than optimal viability or viable recovery score, the IQA will issue a Potential Issue Alert (PIA) email to the labs and applicable technician(s) signifying 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 laboratory.

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.
     - 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.
- 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.

Any technician who has received an Unsatisfactory score will not be allowed 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 if there are no currently approved processors.

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.

The Processing Lab Laboratory Director should also 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 are >1 technicians certified for a laboratory, any technician who has received a satisfactory score on their last submission and who has participated as part of their most recent regularly scheduled rotation, is allowed to continue processing PBMCs.**

Each laboratory can find their current list of approved PBMC processors here: https://dhvi.duke.edu/programs-and-centers/immunology-virology-quality-assessment-center/research-programs/programs/immunology-quality-assessment/programs/cryopreservation.

- Select Domestic, for laboratories within the United States, or International, or laboratories outside of the United States.
- For Domestic laboratories: Find your state and select your laboratory.
- For International laboratories: Find your country and select your laboratory.

Only approved PBMC processors will be listed on this website.

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