The Leukapheresis procedure is instrumental in safely obtaining a large number of PBMC samples during a single participant visit. The elevated cost and effort required to collect, process, cryopreserve and ship PBMC samples collected from leukopak products has brought about a heightened need for additional QC measures. ACTG laboratories are required to complete an Initial Leukopak Processing Qualification process, with annual updates, and to maintain successful Near Time Leukopak Processing Quality Control (QC) to be eligible to process these samples for ACTG studies. This document describes the Initial/Annual Leukopak Processing Qualification process and the ongoing Near Time QC requirements.

ACTG Initial or Annual Renewal Leukopak Processing Qualification

First, participation in the Division of AIDS (DAIDS) Immunology Quality Assessment (IQA) PBMC Cryopreservation Proficiency Testing (PT) Program is required for all ACTG laboratories cryopreserving PBMC samples from whole blood (routine and large volume blood draw) and leukopak products for ACTG protocols.

Laboratories must meet the following IQA Cryo PT Program criteria to be eligible to process leukopak products for ACTG protocols:

- The laboratory must maintain an overall combined status of Approved (A) or Provisionally Approved (PA) over the past four quarters. A detailed description of the established performance evaluation is available in the IQA Cryopreservation PT Program Description document located on the IQA website (https://iqa.center.duke.edu/resources/cryopreservation/enrollment).

- In the case that a laboratory receives an overall combined status of On Probation (OP), the IQA will be available for assistance through the Investigation Report (IR) form process. The laboratory must improve their overall combined status to an Approved (A) or Provisionally Approved (PA) by the means of the sample resubmission process.

- A laboratory that is currently participating in the IQA Cryo PT Program should express interest in becoming qualified to process ACTG leukopak products to the ACTG leadership group (actg.labcenter@fstrf.org) prior to requesting initial qualification.

Second, a laboratory must exhibit the ability to accurately isolate and ship high quality PBMC samples collected from a leukopak product in accordance with the ACTG/IMPAACT PBMC Isolation from Leukapheresis SOP. (https://www.hanc.info/labs/labresources/procedures/Pages/actgImpaactLabManual.aspx).

ACTG laboratories are required to successfully collect, process, and cryopreserve a leukopak product at least once every 12 months to maintain their annual leukopak processing eligibility.

A laboratory that has not processed an on-ACTG-study leukopak product in the past 12 months, will be required to renew their qualification status by collecting an off-study leukopak or purchasing a product from a commercial source (e.g. StemExpress LLC). A commercial leukopak product may be shipped overnight from the source to the processing laboratory, and processing will occur on the following day.

- A leukopak collected for initial or renewal qualification purposes may be from a HIV negative donor.

- The laboratory should collect or purchase enough product to cryopreserve a minimum of 85 vials at 50 million cells/mL (i.e. 4.5 billion cells). This enables the laboratory to be familiar with the effort and workflow needed to process a whole leukopak product.

- The entire cost of the initial or renewal qualification process, including the collection/purchase of the leukopak and shipment to the IQA, is the laboratory's or site's responsibility.

- The laboratory must ship a total of 8 leukopak PBMC aliquots at -80°C (4 samples from the beginning, and 4 samples from the end of the processing) on dry ice to the IQA for evaluation. The laboratory must copy the ACTG Network Lab Center (ACTG.labcenter@fstrf.org) on notifications to the IQA for initial or renewal leukopak processing qualification shipments.

- The leukopak qualification samples should be entered into LDMS under the IQA group; a cryopreservation assay test is not required for leukopak qualification samples.

- If timing of the leukopak processing allows, qualification samples may be shipped with regular IQA Cryo PT Program submission samples. The leukopak samples should be separated, clearly marked and included in a separate LDMS batch, shipping manifest and e-shipping file. LDMS shipping manifest and shipping batch.

- After receipt, the IQA will store the leukopak qualification samples in LN2 vapor phase freezer for a minimum of 2 days before evaluation. Leukopak samples will be evaluated per leukapheresis performance standards.

- The IQA will notify the sending laboratory and the ACTG Network Lab Center of the Initial/Annual Leukopak Qualification Result Report (see Attachment #1).

- Laboratories are given a maximum of 3 attempts to successfully complete the initial/annual leukopak qualification process.

Near-Time Leukapheresis Processing Quality Control (QC)

Near-Time Leukapheresis QC is required for each leukopak processed in support of an ACTG protocol to ensure the quality of cryopreserved PBMC samples. The leukopak processing laboratory is required to submit leukopak samples to the IQA for evaluation. The IQA will evaluate samples collected from each ACTG leukopak processed as part of the Near Time Leukapheresis QC program.

- Within 4 weeks of processing an ACTG leukopak, see LPC for specific guidance, the laboratory must ship a total of 4 leukopak samples at -80°C (2 samples from the beginning and 2 samples from the end of the leukopak aliquoting) on dry ice to the IQA for evaluation. Note that some protocols may specify a shorter window for shipment of near-time QC samples.

- If timing of the processing allows (i.e., complying with the "maximum-5-weeks at -80C rule"), Near-Time Leukapheresis QC samples may be sent with regular IQA PBMC Cryo PT Program submission samples. The samples should be separated, clearly marked and included in a separate LDMS batch, shipping manifest and e-shipping file. (This is the only case in which the quarterly IQA PBMC Cryo PT Program samples may be shipped using the ACTG shipping account number.)

- After receipt, the IQA will store the Near-Time Leukapheresis QC samples in LN2 vapor-phase freezer for a minimum of 2 days before evaluation. The leukopak samples will be evaluated per leukapheresis performance standards.

- The IQA will notify the sending laboratory and the ACTG Network Lab Center of the Near-Time Leukapheresis QC Result Report (see Attachment #2).

- The use of the ACTG shipping account numbers for FedEx and World Courier is allowed for ACTG protocol Near-Time Leukapheresis QC shipments to the IQA. Use of the ACTG account is only intended for Near-Time Leukapheresis QC protocol specific shipments and not for leukopaks purchased in an effort to begin or maintain annual leukopak qualification, or IQA PBMC Cryo PT Program shipments. Air waybills should be clearly marked to reflect the Near-Time Leukapheresis QC.

Leukapheresis Performance Standards

All leukopak samples will be evaluated per leukapheresis performance standards established by the IQA and ACTG leadership. During the "Initial or Annual Renewal Leukopak Processing Qualification", the IQA will evaluate 8 leukopak samples (4 from the beginning and 4 from the end of the leukopak), and report the best possible results for a total of 4 samples. During the Near Time Leukapheresis QC process, the IQA will evaluate 4 leukopak samples (2 from the beginning and 2 from the end of the leukopak), and report the best possible results for a total of 2 samples.

- Each leukopak sample reported by the IQA will receive a score for both the viability and viable recovery percentage; see Figures 1.0 and 2.0.

Figure 1.0 - Scoring for Viability percentage, a score less than 2 is considered out of range.

Viability %	Performance Score
85% to 100%	2
70% to 85%	1
<70%	0

Figure 2.0 - Scoring for Viable Recovery percentage, a score less than 2 is considered out of range.

Viable Recovery %	Performance Score
80% - 120%	2
50% to 80%	1
120% to 150%	1
<50%	0
>150%	0

- The viability scores for each leukopak sample are combined to yield the viability status; see Figure 3.0.

Figure 3.0 - Combined Viability Score.

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

The viable recovery scores for each leukopak sample are combined to yield the viable recovery status; see Figure 4.0.

Figure 4.0 – Combined viable recovery status.

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

- The viability status and viable recovery status are combined to yield an overall processing status. The overall combined processing status is determined by the lower of the viability and viable recovery statuses, see Figure 5.0.

Figure 5.0 – Overall Processing Status.

Viability % Status	Viable Recovery % Status	Overall Processing 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)

- The overall combined processing status determines a laboratory's eligibility to begin or continue to process leukopak products for ACTG protocols, see Figure 6.0.

- Laboratories that receive an out of range performance score for one or more leukopak samples (see Figures 1.0 and 2.0) are required to submit an ACTG Leukopak Investigation Report (IR) form to the IQA for review (see Attachment #3). The ACTG Leukopak IR form should be completed within 2 weeks of receiving the result report. The IQA will communicate directly with the laboratory and work to resolve the deficiency.

- Laboratories that receive an overall combined processing status of Provisionally Approved (PA) or On Probation (OP), are required to update the LDMS condition code from SAT to either:
  -VRU = Viable recovery may be outside expected parameters (higher or lower)
  -VPL = Viability percent may be less than the expected parameter

If the aliquots are at BRI (or at a testing lab), the processing lab must request BRI (or the testing lab) to update the condition codes appropriately. The Processing Lab should inform the IQA when the condition codes have been updated within 2 weeks of receiving the result report.

- Laboratories must receive no less than an overall combined processing status of Approved (A) or Provisionally Approved (PA). Laboratories with substandard results, On Probation (OP), will not be eligible to process leukopak products for ACTG protocols.

Figure 6.0 – Summary of overall processing status specifications.

Overall Combined Processing Status of Annual Qualification or Near Time Leukapheresis QC Leukopak samples:	If any sample received a score of 0 or 1, required to submit an ACTG Leukopak IR Form?	For Near Time QC LPk samples, update the LDMS condition code from SAT to either: • VRU = Viable recovery may be outside expected parameters (higher or lower) • VPL = Viability percent may be less than the expected parameter	Eligible to start or continue leukopak processing for new ACTG protocols?	Required to re-submit an additional annual leukopak qualification round (3 attempts allowed)?	Notes:
A	Yes	No	Yes	No	• The laboratory is exhibiting excellent leukopak processing practices. • Completion of an ACTG LPk IR form is required for any sample that receives an out of range score for either viability or viable recovery.
PA	Yes	Yes	Yes	No	•Complete the ACTG LPk IR form and work with the IQA to improve leukopak processing performance. • Update LDMS condition codes to reflect the out of range results.
OP	Yes	Yes	No	Yes	• Complete the ACTG LPk IR form and work with the IQA to improve leukopak processing performance. • Update LDMS condition codes to reflect the out of range results. • The laboratory is not eligible to begin or continue processing leukopak products for ACTG protocols.

Attachment #1 – Initial or Annual Renewal Leukopak Qualification Result Report Template

Duke University School of Medicine
Immunology Quality Assessment Laboratory

To: Site

From: IQA Cryopreservation PT Program

Re: Leukapheresis Annual Qualification Results (Site, LDMS #___; GLOBAL SPEC & Number; Received Date)

Date of Report:

The leadership of the AIDS Clinical Trials Group (ACTG) require participation in a near-time proficiency testing program to evaluate a laboratory's ability to reliably cryopreserve viable PBMCs obtained from a leukapheresis product while conducting a protocol. Performance in this program is currently reviewed by IQA Cryopreservation PT Program.

The viability and viable percent recovery for samples was assessed and results are provided in this report.

The Immunology Quality Assessment Contract Laboratory (IQA) at the Duke Human Vaccine Institute received four cryopreserved PBMC aliquots processed a leukapheresis product from a single participant. Selected vials were thawed, and viability percentage and percent viable cell recovery were determined. Laboratory performance is provided below in Table 1.

Table 1: Percent Viability and Viable Recovery

# of vials thawed	Global Spec ID	PID	Viability (%)	Viability Score	Viability Status	Viable Recovery (%)	Viable Recovery Score	Viable Recovery Status	Combined Status

The staff at the IQA Center is available for technical consultation concerning these results. Contact Raul Louzao at 919-684-5861, raul.louzao@duke.edu, or Sarah Keinonen, sarah.keinonen@duke.edu.

Thank you,
IQA Cryopreservation PT Program

Attachment #2 – Near Time Leukapheresis QC Result Report Template

Duke University School of Medicine
Immunology Quality Assessment Laboratory

To: Site

From: IQA Cryopreservation PT Program

Re: Leukapheresis Near-Time Cryopreservation QA Results (Site, Lab LDMS #___; Global Spec; Received Date)

Date of Report:

The leadership of the AIDS Clinical Trials Group (ACTG) require participation in a near-time proficiency testing program to evaluate a laboratory's ability to reliably cryopreserve viable PBMCs obtained from a leukapheresis product while conducting the (ACTG Study) protocol.

The viability and viable percent recovery for selected samples was assessed and results are provided in this report.

The Immunology Quality Assessment Contract Laboratory (IQA) at the Duke Human Vaccine Institute received four cryopreserved PBMC aliquots processed a leukapheresis product from a single (ACTG Study) study participant. Selected vials were thawed, and viability and percent viable cell recovery were determined. Laboratory performance is provided below in Table 1.

The data obtained from the vials sent to the IQA by Site, Lab LDMS #___ are shown below in Table 1.

Table 1: Percent Viability and Viable Recovery

# of vials thawed	Global Spec ID	PID	Viability (%)	Viability Score	Viability Status	Viable Recovery (%)	Viable Recovery Score	Viable Recovery Status	Combined Status

The staff at the IQA Center is available for technical consultation concerning these results. Contact Raul Louzao at 919-684-5861, raul.louzao@duke.edu, or Sarah Keinonen, sarah.keinonen@duke.edu.

Thank you,

IQA Cryopreservation PT Program

Attachment #3 – ACTG Leukapheresis Investigation Report (IR) Form

ACTG LEUKAPHERESIS EXTERNAL QUALITY ASSURANCE PROGRAM, INVESTIGATION REPORT FORM

ACTG Leukapheresis External Quality Assurance Program, Investigation Report Form: Leukapheresis Qualification

EXTERNAL QUALITY ASSURANCE (EQA) INFORMATION

Note: Please complete all sections of this investigation report except where stated for "ACTG Network Use Only". Submit the report to the IQA (sarah.keinonen@duke.edu) and ACTG Laboratory Science Group (ACTG.labcenter@fstrf.org).

CRS #:		EQA Provider: IQA	
Laboratory Name:			
LDMS #:			
EQA Name:	IQA/ACTG Leukapheresis Qualification	Leukapheresis Processing Technicians (First and Last Name):	
		1.	4.
		2.	5.
		3.	6.
Date Leukapheresis was Performed:			
PID			
Visit			
Leukopak Processing SOP# and Version Number:			
Leukopak LDMS#			
Date Leukapheresis PBMC Aliquots were Submitted to the IQA for EQA Evaluation:			
Previous Leukapheresis Processing Problems (If yes, explain):			
Investigation Performed By (Name, Title, Email):		Date:	

Protocol Sample Processing Incident/Deviation (completed by IQA):

Leukopak PBMC Processing QA					
Unacceptable Leukapheresis EQA Aliquot Identifier	IQA Reported % Viability	Acceptable % Viability	IQA Reported Viable Recovery	Acceptable Viable Recovery Range	Comment
		>85%		80-120%	
		>85%		80-120%	
		>85%		80-120%	
		>85%		80-120%	
		>85%		80-120%	
		>85%		80-120%	
		>85%		80-120%	
		>85%		80-120%	

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ACTG LEUKAPHERESIS EXTERNAL QUALITY ASSURANCE PROGRAM, INVESTIGATION REPORT FORM

ROOT CAUSE ANALYSIS			
PRE-PROCESSING FACTORS:	YES	NO	N/A
1. Did laboratory and site review LPC and coordinate activities? Comments:	☐	☐	☐
2. Was leukopak received by processing lab within 2 hours of collection? Comments:	☐	☐	☐
3. Were transport conditions appropriate to protect leukopak from temperature extremes? Comments:	☐	☐	☐
4. Were any problems noted with the leukopak prior to processing? (e.g., labeling issues, leaking container, obvious clotting) Comments:	☐	☐	☐
5. Were there any other pre-processing factors that may pertain to this investigation? If yes, please explain. Comments:	☐	☐	☐
PROCESSING FACTORS:	YES	NO	N/A
1. Was the leukopak processed in accordance with the ACTG/IMPAACT PBMC Isolation from Leukapheresis Standard Operating Procedure (SOP)? Comments:	☐	☐	☐
2. Were reagents examined for acceptability (*quality, kept at proper storage temperatures, used within defined shelf life, other QC*)? Comments:	☐	☐	☐
2. Was the leukopak material diluted adequately prior to lymphocyte separation? (i.e. diluted with WDR to at least 600mL for a whole leukopak or to 300mL for a half leukopak) Comments:	☐	☐	☐
3. Was an automated cell counter utilized? If yes, provide make and model and verify that all QC was performed and valid prior to use. Please indicate if this is the same system utilized for routine PBMC cryopreservation. Comments:	☐	☐	☐
4. Was a manual (hemacytometer) used to perform the cell counts? If yes, indicate if this is the same system used for routine PBMC cryopreservation. Comments:	☐	☐	☐
5. Were additional dilutions required to obtain valid cell count? Please verify that the final correction factors used to calculate the total cell count were accurate. Comments:	☐	☐	☐
6. Was a PBMC Processing record worksheet utilized in real-time to document reagents, dilutions, calculations, other factors? Comments:	☐	☐	☐
7. Were harvested cells resuspended in CPS in batches to minimize exposure to CPS? Comments:	☐	☐	☐
8. Was gentle mixing of the cells in CPS employed during distribution into aliquot vials? Comments:			

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ACTG LEUKAPHERESIS EXTERNAL QUALITY ASSURANCE PROGRAM, INVESTIGATION REPORT FORM

10. Was processing completed and aliquots in freezer within 8 hours of collection (Leukapheresis procedure stop time)? Comments:	☐	☐	☐
11. Are questionable results reviewed by supervisor and is that review documented? Comments:	☐	☐	☐
12. If applicable, was prior EQA performance for leukapheresis processing reviewed, investigated and problems resolved? Comments:	☐	☐	☐
13. If applicable, was prior EQA performance for routine PBMC processing reviewed, investigated and problems resolved? Comments:	☐	☐	☐
14. Any other processing factors that may pertain to this investigation? Comments:	☐	☐	☐
PROCESSING FACTORS:	YES	NO	N/A
1. Were leukapheresis aliquots shipped and stored appropriately according to temperature requirements to the IQA for this EQA program? Comments:	☐	☐	☐
2. Was the shipment prepared according to ACTG and IQA shipment guidelines for cryopreserved PBMCs? Comments:	☐	☐	☐
3. Was proper temperature of aliquots maintained during shipment (was the laboratory notified by the IQA that temperature control failed during shipment)? Comments:	☐	☐	☐
4. Was the shipment delivered to IQA on time? If not, explain reason for delay. Comments:	☐	☐	☐
5. Any other post-processing factors that may pertain to this investigation? Comments:	☐	☐	☐

INVESTIGATIVE ACTIONS AND ROOT CAUSE

Briefly discuss what actions were taken in this investigation:

What do you believe is/are the primary cause (s) of this problem?

Type of Error:

- ☐ Methodological
- ☐ Technical
- ☐ Clerical
- ☐ EQA evaluation problem
- ☐ Other (explain) _____

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ACTG LEUKAPHERESIS EXTERNAL QUALITY ASSURANCE PROGRAM, INVESTIGATION REPORT FORM

Future Preventative Measures/Actions
Discuss how you will prevent this problem from occurring in the future:

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ACTG LEUKAPHERESIS EXTERNAL QUALITY ASSURANCE PROGRAM, INVESTIGATION REPORT FORM

Investigative Report Prepared by:

Name/Title	Date	Signature
PI Name/Title	Date	Signature

FOR ACTG NETWORK USE ONLY- IQA REVIEW

COMMENTS:

		☐	Acceptable and complete Investigation.
Name/Title	Date	☐	Investigation is incomplete. See comments.

Study Impact

If applicable, were study participant results assessed for adverse effects?

If applicable, review participant results, amend results and notify the following---physicians, study staff and network representatives.

Comments: