



MEMORANDUM

DATE: June 23, 2017
TO: Network Clinical Trial Units
FROM: Laboratory Focus Group (LFG)
SUBJECT: Reminder Notice for the IQA Cryopreservation Proficiency Testing Program

Sites/Labs Participation

The use of cryopreserved Peripheral Blood Mononuclear Cells (PBMC) for immunologic assays has increased in recent years with the expansion and diversification of sites participating in DAIDS-supported network studies. The utility of cryopreserved PBMC for efficient immunological assays is predicated on their viability and functional ability in vitro.

The leaderships of the AIDS Clinical Trials Group (ACTG) and the International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) require laboratories that cryopreserve PBMCs for network protocols to participate in the Immunology Quality Assessment (IQA)-administered quarterly proficiency testing program to determine their ability to reliably freeze viable PBMCs. Performance in this program is currently reviewed by the LFG.

If your site will not be able to submit samples this iteration of the program, please send a message to Raul Louzao (raul.louzao@duke.edu) or Sarah Keinonen (sarah.keinonen@duke.edu) no later than **September 22, 2017** with an explanation of your circumstances. In your message, please include the specific ACTG/IMPAACT site name and LDMS Lab number unable to participate. Based on LFG recommendation, the network(s) will determine whether or not your lab will be placed “on hold” for a maximum of two rounds of IQA testing. While your lab is “on hold” it is not allowed to cryopreserve PBMC specimens for ACTG or IMPAACT protocols.

Participating Labs:

We will start receiving samples the week of **September 11, 2017**.
Samples will be considered LATE after **September 22, 2017**.

Prepare Samples

1. Identify two (2) blood donors and obtain their consent:
 - a. Submission of HIV-positive PBMC samples is no longer required; laboratories may now submit HIV-negative PBMC samples for scoring. In other words, a site may now choose to submit cells from either HIV+ or HIV- donors but must indicate the HIV serostatus of the donor. Labs do not need to provide documentation of the HIV- serostatus.
 - b. Refer to <http://iqa.center.duke.edu/resources/cryopreservation/enrollment> for a sample IRB informed consent form.
2. Make arrangements to obtain blood from two (2) volunteers as early as the week of **August 14, 2017**. (Samples must be stored frozen for at a minimum of 1 week, and a maximum of 5 weeks before shipping to the IQA)
3. From each donor, obtain at least 20mL of blood by standard venipuncture technique. ACD, EDTA or Heparin may be used as the anticoagulant.



4. Isolate PBMC's from each donor according to the Cross-Network PBMC Processing SOP following the protocol in which you currently process PBMCs; this can be downloaded at <http://www.hanc.info/labs/Pages/PBMCSOP.aspx>.
 - a. Determine the cell yield and viability by trypan blue dye exclusion.
 - b. Both % viability and viable cell recovery will be assessed by the IQA after thawing, so an accurate count of total viable cells in each vial is important. For instructions on cell counting methodology refer to <http://iqa.center.duke.edu/resources/cryopreservation/processing>.

5. You should have four aliquots for each donor. Each aliquot should contain a viable cell concentration of **$\sim 5 \times 10^6$ cells per cryovial**, but concentrations as low as 3×10^6 cells per cryovial are accepted.

Note:

- If the total viable cells are less than 20×10^6 cells but greater than 12×10^6 cells, divide the PBMC into four equal aliquots and be sure the LDMS label reflects the actual number of cells per vial.
- If the total viable cells are less than 12×10^6 cells, divide the PBMCs into two equal aliquots and ship to the IQA.
- **DO NOT SHIP VIALS CONTAINING LESS THAN 3×10^6 cells per vial OR VIALS CONTAINING MORE THAN 10×10^6 cells per vial.**

6. **The freezing process and cell storage should be the same as the freezing process and cell storage your lab uses for study participant samples.**

Note: If you cryopreserve PBMC for both ACTG and IMPAACT, you might choose to use one of these options:

- Use the freezing and storage methods you would normally use for the network for which you process the largest number of samples
- Alternate between ACTG and IMPAACT methods from round to round
- Within each round of testing, use ACTG methods for the samples from one donor and IMPAACT methods for the samples from the other donor

7. **Ship to the IQA two aliquots from each donor** and store two back-up aliquots from each donor as your lab would normally store study participant samples. **Please do not send additional aliquots.** The back-up aliquots might be needed for future testing or in-lab investigation.



LDMS

It is **MANDATORY** that all IQA Cryopreservation and Viability samples be logged in, resulted and shipped through LDMS. Please follow the steps outlined below to ensure that the data has been entered properly. For additional information, please reference the LDMS User Manual sections listed for each step. More detailed instructions for the IQA Cryopreservation Panel Procedure can be found on the IQA website at <https://iqa.center.duke.edu/resources/cryopreservation/processing>. If you have any questions regarding the LDMS program, please contact LDMS user support at 716-834-0900 ext. 7311 or email ldmshelp@fstf.org.

Step 1: Log the samples into Specimen Management.

Samples must be logged into the Specimen Management module as group IQA with the PID or donor identifier entered into the ID1 field. For each aliquot, enter the cell count for the volume with CEL as the derivative and the volume unit. The IQA Cryopreservation and Viability assay should be ordered in the Test Setup tab of Specimen Management for each aliquot.

Step 2: Generate Labels for IQA Specimens.

Choose the IQA Barcode Label or use the Labels module for the IQA specimens that will print out the ID1, LDMS specimen #, cell volume, specimen date and derivative type for each label. Make sure the IQA label shows (IQA group provides a fixed IQA Barcode label):

Specimen ID (donor #)
Global Spec ID (LDMS #)
Specimen Date (draw date)
PRIM/ADD/DER (cell)
Cell Volume/ml

Step 3: Complete the IQA Cryopreservation and Viability Data Entry Screen

Go into the Assay module and select the IQA Cryopreservation and Viability assay from the Immunology tests that are listed. Select a new run and >search for your specimens in the Filters/Criteria tab. Select your specimens and complete all fields on the Results tab. Print out patient reports for your records.

Step 4: Create a Shipping Disk and Manifest Report

In the Shipping module, search for your IQA samples and create a shipping disk to be sent to the IQA **Lab 213**. Alternately, you can create an LDMS Shipping Batch file to be emailed to Lab 213. **These files (from either the shipping disk or shipping Batch file) will need to be imported into Lab 213 and are required to proceed.** Print out and include a copy of the manifest report in your shipment.



Shipping Instructions

Make arrangements to ship the samples on dry ice, on Monday, Tuesday or Wednesday during the week of **September 11, 2017**.

You can find detailed shipping instructions in the document entitled [AIDS Clinical Trials Network \(ACTN\) Instructions for Overnight Shipments](https://iqa.center.duke.edu/resources/cryopreservation/shipping) at <https://iqa.center.duke.edu/resources/cryopreservation/shipping>.

You can also view a cross-Network shipping training video online at <https://www.hanc.info/labs/labresources/procedures/Pages/actnShippingDemo.aspx>. Please note that for the IQA Cryopreservation Proficiency Testing Program, you will be shipping a Category B Biological Substance.