Infectious Diseases Clinical Research Consortium

16 PBMC Proficiency and Immunology Quality Assurance (IQA) Process

16.1 Purpose

The purpose of this section of the IDCRC MOP is to:

(1) describe the process for enrollment and continued participation in the Duke Immunology Quality Assurance (IQA) PBMC Cryopreservation Proficiency Testing (PT) Program.
(2) describe the measures taken by the Duke IQA program when performance issues are identified;
(3) describe the measures taken by the IDCRC Laboratory Operations Unit (LOU) when performance issues are identified either by the Duke IQA program from testing PBMC collected and processed for quarterly testing or by the endpoint laboratories upon evaluating PBMC collected under IDCRC protocols; and
(4) detail the responsibilities of the VTEU PI and affiliated specimen processing lab (SPL) staff regarding PBMC specimens processed at their site for IDCRC studies to ensure acceptable quality, integrity, and reliability of endpoint laboratory assay data resulting from testing these PBMC specimens.

IQA evaluations represent a proxy for the expected quality of PBMC specimen processing for protocol specimens at a given SPL. The LOU and protocol stakeholders (e.g., DMID, developers, protocol teams) rely heavily on the IQA evaluations to determine the eligibility of laboratories for participation in future protocols as well as escalating potential quality concerns demonstrated during an active protocol. Escalated concerns will be communicated to the associated SPL, the associated VTEU PI and clinical site PI (if applicable), protocol team leadership, and other stakeholders. Timely and pertinent communication and corrective actions will be key to the uninterrupted participation of a given laboratory in current and future IDCRC studies.

16.2 Scope

Laboratories that will be participating in an IDCRC protocol involving PBMC cryopreservation are required to participate and remain in good standing (see description of statuses below) in the Duke IQA PBMC Cryopreservation PT Program. The full program description can be found here. This program measures both viability and viable recovery (immediately after thawing and with an optional overnight incubation) of PBMC and assigns a score to each parameter in order to: (1) assess a laboratory's ability to provide quality PBMC for use in IDCRC protocols; (2) respond quickly to marginal or poor performance; (3) give laboratories sufficient opportunity to improve performance before employing mitigating actions; (4) provide an incentive for laboratories to continue participation and high performance; and (5) provide data to networks for choosing labs and/or specimens for studies. Performance issues of SPLs may also be identified by endpoint

assay labs upon evaluating PBMC collected under IDCRC protocols. The LOU has oversight in addressing these issues and will work in partnership with the SPLs in resolution.

16.3 Procedure

16.3.1 Participation in IQA and resolution of performance issues

Enrollment: Once sites have been selected to participate in an IDCRC protocol involving cryopreservation of PBMC, the LOU will email each VTEU or affiliated site to inform them of the process for enrollment and participation in the Duke IQA program.

Fast-Track for initial approval and re-approval: In order to quickly become eligible to cryopreserve PBMC for IDCRC protocols, an SPL must submit the equivalent of two rounds of PBMC PT samples. The related blood specimens should be collected in the blood collection tubes to be used in the respective protocol (e.g., BD Vacutainer® CPT™ Mononuclear Cell Preparation Sodium Citrate Tubes), PBMC should be isolated and cryopreserved by following the SOP implemented in the protocol, and blood should be drawn and frozen on at least two different days. The SPL must notify the IQA PBMC Cryopreservation PT Program in advance for the samples to be evaluated out of the normally scheduled PT track. If the evaluation report indicates acceptable viability and viable recovery scores, the SPL will be deemed "Satisfactory" status and may participate in the IDCRC protocol. The Fast-Track process may take as long as two months. An SPL that receives a "Not Approved" status because it did not submit samples for two rounds of testing may not use the fast track for re-approval without special permission from the LOU and the IQA program. In some rare occasions (e.g., during a pandemic) and with pre-approval by the LOU, an SPL may process blood for PBMC cryopreservation for an IDCRC protocol while awaiting a "Satisfactory" status from the IQA program.

Continued Participation: Each SPL that will cryopreserve PBMC for an IDCRC protocol must submit two aliquots of cryopreserved PBMC from each of the two blood donors for ongoing quarterly IQA evaluation. Blood should be collected in the blood collection tubes to be used in the respective protocol (e.g., BD Vacutainer® CPT™ Mononuclear Cell Preparation Sodium Citrate Tubes) and PBMC should be isolated and cryopreserved by following the SOP implemented in the protocol; if the laboratory is contributing to multiple protocols that require differing collection containers and/or differing separation methods, the laboratory should use the collection container and/or the separation method most frequently used. Any questions surrounding the appropriate collection containers or methods must be shared with the LOU. SPL should NOT submit additional aliquots unless directed by the IQA program. If there are two or more technicians cryopreserving PBMC for a given IDCRC protocol, the technicians should rotate who submits samples each quarter; **each technician must submit a minimum of one set of samples each year.**

An aliquot from each donor is analyzed by the IQA program and receives a score ranging from 0 to 2 for viability status and viable recovery status. The scores from each donor are combined to determine the overall status:

- Satisfactory: Viability and viability recovery statuses are acceptable. This is a combined score between 2-4 in both categories.
- Satisfactory with a Potential Issue Alert (PIA): Though the overall status is "Satisfactory," there are concerns about viability and/or viable recovery. The SPL will be issued a PIA indicating that there *may be* an issue that could cause a future "Unsatisfactory" performance status (see next bullet). This is a combined score between 2-4 overall, with one of the scores either 0 or 1.
    - *Actions required by Duke IQA:* Duke IQA program will issue the PIA to the site. No further action will be required by the Duke program once the alert has been received at the laboratory.
    - *Actions required by LOU:*
        - *One PIA:* If one PIA is received by a laboratory, the LOU will request a formal Investigative Report (IR) to be submitted to the LOU within five working days of the initial request. DMID and the IDCRC Leadership will also be notified. After a review of the completed IR, overall laboratory performance, and the results contained within the most recent IQA report, the LOU *may* set up a call with the SPL to discuss the IR and to further assist with process troubleshooting. A summary of the call will be shared with appropriate stakeholders (e.g., VTEU PI, clinical site PI if applicable, IDCRC Leadership, DMID).
        - *Two or more consecutive PIAs:* If an SPL receives two consecutive PIAs, the LOU will consider the SPL as posing a moderate to high risk to any active protocols for which PBMC are being managed by the laboratory. The LOU will inform the protocol chairs of each study in which the SPL is currently participating and/or being considered for in the future; DMID, the IDCRC Leadership, and other key stakeholders will be notified. The SPL and VTEU PI (or clinical site PI if applicable) will be responsible for thoroughly investigating and fully addressing the PIA within five working days of notification and providing an action plan for process improvement to the LOU to avoid possible suspension of research activities. The LOU will schedule a call with the laboratory to discuss investigation findings and related changes implemented within the laboratory to address the root cause(s) of performance issues identified. Other protocol actions will depend on the status of assays utilizing PBMC within each study as described below:

- If the PBMC are being used for *primary* endpoint assays, the protocol team may consider suspending enrollment at that SPL until it demonstrates the ability to cryopreserve PBMC of acceptable quality. In addition, the protocol team may decide to suspend the collection of specimens for PBMC isolation for participants already enrolled. Another but less likely option would require the transfer of processing activities to another Duke IQA-qualified SPL; the transfer must be approved by the LOU and protocol stakeholders. If approved, the impacted clinic and laboratories would be responsible for all operational components related to the transfer (e.g., planning, standard operating procedures).
- If the PBMC are being used for *secondary* or *exploratory* endpoint assays, the protocol leadership will consider continuing enrollment at the affiliated site while improvements to PBMC processing are underway.
- If PBMC are being collected solely for secondary research, PBMC collection and processing may be stopped at the site, although visits would continue without collection of these specimens. If collection and processing PBMC collected for secondary research is halted, sites should not draw down funds for specimens not collected.
- Until the issue is resolved, PBMC specimens processed during the three months preceding the initial PIA will be considered for flagging purposes depending upon the type of issue, severity of the issue, observable trends, and overall performance of the laboratory. Any flagging of specimens will be proposed to protocol, sponsor, and IDCRC Leadership. If a consensus for flagging is reached, the LOU will work with the protocol's statistics and data management organization (SDMO) to accomplish the flagging. Depending on conditions, flagged specimens may be withheld from assays, or assay data may not be included in final study reports.

- Unsatisfactory: Viability and viability recovery statuses are not acceptable. This is a combined score of 0-1 in either category.
  - *Actions required by Duke IQA:* The SPL must (1) submit an Investigative Report (IR) to the IQA program within 5 working days of receipt of the "Unsatisfactory" status report; (2) submit the equivalent of one round of testing to the IQA program within 4 weeks of "Unsatisfactory" status notification; and (3) receive "Satisfactory" status to continue eligibility. Specimens should be kept frozen on-site for at least 1 week before shipping them to the IQA. If the SPL receives an "Unsatisfactory" status with the resubmission, they will be placed "On Hold" and will be required to requalify for the Duke IQA program.

- *Actions required by LOU:* The LOU will inform the protocol chairs of each study in which the SPL is currently participating and/or being considered for in the future; DMID, IDCRC PIs, and other key stakeholders will also be notified. The LOU will schedule a call with the laboratory to discuss investigation findings and related changes implemented within the laboratory to address the root cause(s) of performance issues identified. Other protocol actions will depend on the status of assays utilizing PBMC within each study as described below:
    - If the PBMC are being used for *primary* endpoint assays, the protocol team may consider suspending enrollment at that SPL until it demonstrates the ability to cryopreserve PBMC of acceptable quality. In addition, the protocol team may decide to suspend the collection of specimens for PBMC isolation for participants already enrolled. Another but less likely option would require the transfer of processing activities to another Duke IQA-qualified SPL; the transfer must be approved by protocol stakeholders. If approved, the impacted clinic and laboratories would be responsible for all operational components related to the transfer (e.g., planning, standard operating procedures).
    - If the PBMC are being used for *secondary* or *exploratory* endpoint assays, the protocol team will consider continuing enrollment at the affiliated site while improvements to PBMC processing are underway, and the SPL is back in good standing with the Duke IQA program.
    - If PBMC are being collected solely for secondary research, PBMC collection and processing may be stopped at the site, although visits would continue without collection of these specimens. If collection and processing PBMC collected for secondary research is halted, sites should not draw down funds for specimens not collected.
    - Until the issue is resolved, specimens processed during the three months preceding the “Unsatisfactory” status will be considered for flagging purposes. Any flagging of specimens will be proposed to protocol, sponsor, and IDCRC Leadership. If a consensus for flagging is reached, the LOU will work with the protocol’s SDMO to accomplish the flagging. Depending on conditions, flagged specimens may be withheld from assays, or assay data may not be included in final study reports.
    - If the PBMC resubmission to the Duke IQA program in response to “Unsatisfactory” status is again problematic, the LOU will conduct a phone interview with the SPL personnel within ten business days of awareness to assist in the identification of root cause contribution(s) to the cell yields and/or viabilities. Additionally, all specimens processed during the three months preceding the initial “Unsatisfactory” submission will be flagged in their respective study database until a

"Satisfactory" submission is achieved. At the discretion of the LOU and site PI, the LOU may request an on-site visit of an LOU representative to assist the SPL with improving their PBMC cryopreservation and handling techniques.

- Not Approved: The SPL was unable or unwilling to improve performance after receiving notice that it was in "Unsatisfactory" status and is no longer eligible to perform PBMC cryopreservation for IDCRC protocols.

Continued approval: To maintain approval to cryopreserve PBMC for IDCRC protocols, each SPL must maintain a "Satisfactory" status with the Duke IQA program. Additionally, the SPL must also continue to provide acceptable PBMC for use in protocol-specific assays. Failure to do so will require the SPL to identify and address any issues appropriately and in a timely manner. Further protocol-specific actions will be determined by the protocol, sponsor, and IDCRC leadership on a case-by-case basis.

Withdrawal/Removal: An SPL that moves to "Not Approved" status due to missing samples (i.e., the SPL did not submit samples and did not work with the IQA to submit late samples) will be contacted by the LOU to discuss continued participation in the IQA program. An SPL may request that proficiency testing for PBMC cryopreservation be put "On Hold" due to operational circumstances (e.g., personnel problems, change of instrument, no active protocols) for up to two quarters. For requests beyond two quarters, sites will need to requalify with the IQA program to resume participation. An SPL may not perform PBMC cryopreservation in any IDCRC protocol requiring viable PBMC, or serve as a backup laboratory, while in an "On Hold" status.

Backup Laboratory: If an SPL is in "Not Approved" status, the clinical site may identify an IQA-certified backup laboratory in order to continue to cryopreserve PBMC for an IDCRC protocol(s). The transfer must be approved by the LOU, IDCRC Leadership and DMID while taking funding of any such transfer into consideration. If approved, the impacted clinic and laboratories would be responsible for all operational components related to the transfer (e.g., planning, standard operating procedures).

16.3.2 Resolution of performance issues identified by endpoint assay laboratories.

Once PBMC collected under IDCRC protocols are provided to endpoint laboratories to evaluate in their specialized assays, issues related to performance of the SPLs may be identified (e.g., low PBMC recovery, low PBMC viability). When such issues arise and are communicated to the LOU, the LOU will notify the VTEU PI and DMID and work in partnership with the SPL to resolve the issue. The LOU may request that the SPL investigate the issue to determine the root cause and the SPL is responsible for conducting a thorough investigation and reporting the findings to the LOU in a timely manner. If needed, the LOU will schedule an ad hoc call with the SPL to discuss the findings and strategize with the SPL on a resolution. Depending on the issue and/or root cause, a CAPA may be required. The impacted specimens may be considered for flagging in the

database and the specimens may be withheld from assays, or assay data may not be included in final study reports.