Infectious Diseases Clinical Research Consortium

16. PBMC Proficiency and Immunology Quality Assurance (IQA) Process

16.1. Purpose

The purpose of this section of the Infectious Diseases Clinical Research Consortium (IDCRC) Manual of procedure (MOP) is to:

(1) Describe the process for enrollment, approval, and continued participation in the Duke Human Vaccine Institute, Vaccine and Treatment Evaluation Unity (VTEU) Immunology & Virology Quality Assessment Center (IVQAC) Cryopreservation Proficiency Testing (PT) Program (hereafter referred to as the IVQAC Program).
(2) Describe the measures taken by the IVQAC 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 IVQAC Program during quarterly testing of peripheral blood mononuclear cells (PBMC) submitted by participating sites or by the endpoint laboratories upon evaluating PBMC collected under IDCRC protocols. Coordination with other entities on oversight and investigation of proficiency results for VTEU participating in both IDCRC and other networks is also described.
(4) Detail the responsibilities of the VTEU Principal Investigator (PI) and affiliated specimen processing laboratory (SPL) staff regarding PBMC processed at their site for IDCRC studies to ensure acceptable quality, integrity, and reliability of endpoint assay data resulting from testing these specimens.

IVQAC Program evaluations represent a proxy for the expected quality of PBMC collected under a clinical protocol at a given SPL. The LOU and protocol stakeholders (e.g., DMID, developers, protocol teams) rely heavily on these evaluations to determine the eligibility of laboratories for participation in future protocols as well as to identify potential quality concerns of PBMC collected under an active protocol. PBMC quality concerns will be communicated to the associated SPL, the associated VTEU PI and clinical site PI (if applicable), protocol team leadership, and other stakeholders as needed. Timely and pertinent communication and corrective actions will be key to the uninterrupted participation of a given SPL 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 satisfactory status (see description of statuses below) in the IVQAC Program. The full program description can be found on the IVQAC website:

https://dhvi.duke.edu/programs-and-centers/immunology-virology-quality-assessment-center/research-programs/immunology-4

The 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 to:

(1) Assess a SPL's ability to provide quality PBMC for use in IDCRC protocols.
(2) Respond quickly to marginal or poor performance.
(3) Allow SPLs sufficient opportunity to improve performance before employing mitigating actions.
(4) Provide an incentive for SPLs to continue participation and high performance.
(5) Provide data to networks for choosing SPLs and/or specimens for studies.

Performance issues of SPLs may also be identified by endpoint assay laboratories 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. Parameters that are analyzed in the IVQAC Program

Viability: Viability is a measurement of the proportion of PBMC in the specimen that are alive. Viable cells are required to perform successful functional analyses.

Viable Recovery: Viable recovery is a comparison between the number of PBMC cryopreserved at the SPL and the number of viable PBMC retrieved after thawing. A low viable recovery indicates that there may not be enough cells to complete the required analyses. Inflated viable recovery may indicate courting errors or unequal distribution of cells among all aliquots.

16.3.2. Enrollment in the IVQAC Program

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 IVQAC Program. Each site will be responsible for identifying the personnel who will be involved in participation in the IVQAC Program.

Initial Approval: Newly enrolling laboratories must have **each** processor submit **2 PBMC aliquots** from **1 donor** to the IVQAC Program. If more than one processor from a SPL is participating in the program, PBMC from separate donors should be submitted and PBMC collection and freezing should occur on different days. A maximum number of four processors per lab are allowed to be certified. If more than 4 processors are needed for a certain IDCRC protocol, the LOU, IDCRC LG and the SPL will discuss possible options to expand the number of processors to support the protocol.

Donor 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, NaHep or ACD tubes) and processed following the SOP implemented in the protocol. If the SPL is contributing to multiple protocols that require different collection containers and/or processing methods, the

SPL should use the collection container and/or the processing method most frequently used. Any questions about the appropriate collection containers or methods must be directed to the LOU. The SPL should **NOT** submit additional aliquots unless directed by the IVQAC Program.

If the IVQAC evaluation report indicates acceptable viability and viable recovery scores ("Satisfactory" status), the SPL and corresponding processor(s) will be enrolled to participate in the quarterly rounds of the IVQAC Program, which will begin with the next scheduled PT quarter. The IQA dates can be found on the program's website and will be communicated by the IVQAC Program. Any processor who receives an "Unsatisfactory" score (inacceptable viability and viable recovery) must resubmit PBMC for evaluation until a satisfactory score is received.

For IDCRC protocols involving cryopreservation of PBMC, the SPL and corresponding processors will need to initially achieve and maintain a "Satisfactory" score during participation in the IVQAC Program. If the SPL and corresponding processor is deemed "Satisfactory with a Potential Issue Alert (PIA)", they are allowed to participate in the IDCRC protocol, but the SPL would be required to investigate and submit an IDCRC-Investigative Report (IDCRC-IR) to the LOU as outlined in section 16.3.4.3. If the IQA result by a processor in the quarterly round is deemed "Unsatisfactory", the SPL will need to submit an IVQAC IR and the processor will need submit two PBMC aliquots from 2 separate donors to the IVQAC within 4 weeks of receiving the results report. The results from this re-submission may not be available for up to two months (unofficial timeframe provided by IVQAC). PBMC processing by this processor for active IDCRC protocols may be paused until a "Satisfactory" status is achieved (see section 16.3.4.4). The impact of the IVQAC scoring results and subsequent corrective actions by the SPLs might differ depending on the IDCRC protocol endpoints where PBMC are used for. While evaluating the score and corrective action it should be taken into consideration if PBMCs are used for primary, secondary or exploratory measurements.

An SPL/processor that does not submit specimens for enrollment and continuous approval may not participate in an IDCRC protocol without special permission from the LOU and other stakeholders. 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 IVQAC Program. All exceptions will be recorded in a note to file.

Any discrepancy between the IVQAC Program description and the IDCRC MOP or any issue pertaining to PBMC processing at VTEU SPL that are outside the scope of the IVQAC program will be resolved after discussion with the IDCRC LG and DMID. For example, the IVQAC Program description notes that processors from each lab must be participating in quarterly rounds of PT and maintain a satisfactory score in each round to be able to process PBMC for protocols. While this is generally true for IDCRC protocols, there may be instances where a processor who does not meet these criteria is needed to support an IDCRC protocol due to high workload. For any procedural differences, please refer to and follow the guidance outlined in this MOP. For any additional clarifications, please contact IDCRC.LOU@fredhutch.org.

16.3.3. Participation in the IVQAC Program

Once a laboratory has completed the initial qualification process, they will proceed with quarterly submissions as per IVQAC schedule. Each SPL should have several qualified staff members capable of processing PBMC. A maximum of 2 processors are allowed to submit quarterly. It is the SPL's responsibility to maintain and monitor the certification and participation status of each processor.

If a processor misses 2 consecutive quarters, they will be ineligible to process PBMC until they submit a passing sample. In cases where SPLs only have one staff member capable of PBMC processing, they will submit 2 aliquots from a single donor quarterly. If there are two or more processors cryopreserving PBMC for an IDCRC protocol(s), the SPLs are expected to rotate processors for submitting specimens each quarter; each processor must submit a minimum of one set of specimens every six months. Additionally, each processor assigned to processing PBMC for an IDCRC protocol in pre-development/development must have submitted at least one set of specimens to the IVQAC Program in the past 6 months and prior to study activation. Exceptions are given under certain circumstances out of the SPL control (staff schedule or roster changes) and will need to be discussed with and approved by the LOU.

16.3.4. Scoring Method and Resolutions of Performance Issues

16.3.4.1. The percent viability and viable recovery are assessed during each quarter to determine the processor performance grading. IVQAC receives two aliquots from a single donor for each of the participating processors and one aliquot of those are thawed and analyzed by IVQAC. The quarterly VTEU IVQAC Cryopreservation PT report includes the Day 0 and Day 1 percent viability and viable recovery for each processor and the one aliquot thawed from the single donor (a total of 4 results). Scores range from 0 to 2 for both the percent viability and percent viable recovery. A combination of these scores (ranging from 0-4) will determine the performance grading for each parameter. The status for each aliquot for viability and viable recovery must be "Satisfactory" to receive an overall status of "Satisfactory." If evaluation of aliquot one shows the results not in range, a different IVQAC technician will thaw and evaluate the second aliquot. Between the two aliquots, the best result will be reported by IVQAC to the SPL and received the appropriate status.

16.3.4.2. Satisfactory: Viability and viability recovery statuses are acceptable. This is a combined score between 2-4 in both categories. There are no further actions required by the SPLs and corresponding processors and the laboratories will submit a set of specimens for the next round of quarterly IVQAC Program evaluation.

16.3.4.3. Satisfactory with a Potential Issue Alert (PIA): Though the overall status is "Satisfactory," there are concerns about viability and/or viable recovery. The SPL/processor will be issued a PIA indicating that there *may be* an issue that could cause a future "Unsatisfactory" performance status (see 16.3.4.3). This is a combined score between 2-4

overall, with one of the scores either 0 or 1 (less than optimal viability and/or viable recovery). The below actions are required if a PIA is issued.

Actions required by the IVQAC Program: The IVQAC Program will issue the PIA to the site via email. There is no further action by or response from the IVQAC Program once the informal notice has been issued.

Actions required by IDCRC LOU:

- *One PIA:* If one PIA is received by one or more processors in an SPL, the LOU will request a formal IDCRC Investigative Report (IR) to be submitted to the LOU at **IDCRC.LOU@fredhutch.org** within five working days of the initial IDCRC-IR request. Although this is not an IVQAC Program requirement, it is an IDCRC requirement and thus, **the IDCRC-IR should only be submitted to the LOU and not IVQAC.** The LOU will notify DMID and the IDCRC Leadership of the PIA. If the SPL participates in processing PBMC for other network trials requiring IVQAC participation, the activities will be coordinated between LOU and other entities with the goal of the lab only submitting one IR.
- *Two or more consecutive PIAs:* If one or more processors in 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 processed by the SPL. The LOU will review the results of the last 4 quarters prior to the latest PIA to evaluate the overall risk and support with troubleshooting. The LOU will then request a formal IDCRC-IR to be submitted and will notify DMID and the IDCRC Leadership of the PIA. The LOU *may* inform the chairs of each protocol in which the SPL is currently participating and/or being considered for in the future. 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 submitting the IR.

16.3.4.4. Unsatisfactory: Viability and viability recovery statuses are not acceptable. This is a combined score of 0-1 in either category. The below actions are required if the results are unsatisfactory.

Actions required by the IVQAC Program: The SPL and corresponding processors must (1) submit an IR to the IVQAC Program within five working days of "Unsatisfactory" status notification. The SPL/processor may request a troubleshooting meeting with the IVQAC to identify and resolve possible underlying challenges (i.e., staff training, processing difficulties, counting errors, etc.). The IVQAC will review the IR form for acceptability and provide the finalized IR to the lab, processor(s), and the VTEU; (2) the SPL and corresponding processors must submit **two aliquots** from **two separate donors** within 4 weeks of "Unsatisfactory" status notification; and (3) receive "Satisfactory" status to continue eligibility. If the SPL/processor receives an "Unsatisfactory" status with the resubmission, they will be required to submit PBMC for re-approval (requalification) by the IVQAC Program.

A call will be scheduled by the IVQAC Program to troubleshoot and discuss the result. During this time, the lab and the processor(s) will need to reach out to the VTEU to provide a plan for

recertification and/or continued PBMC processing. At the end of the quarter, the IVQAC provides the VTEU a list of processors that have received an Unsatisfactory performance grading.

Actions required by IDCRC LOU: The LOU will request a copy of the submitted VTEU IVQAC IR form and will stay informed about the re-submission. The LOU will inform the protocol chairs of each study in which the SPL and corresponding processor is currently participating and/or being considered for in the future; DMID, IDCRC Leadership, and other key stakeholders will also be notified. The LOU will participate in the IVQAC call with the SPL and corresponding processor to discuss investigation findings and related changes implemented within the SPL to address the root cause(s) of identified performance issues.

Further actions will depend on the protocol endpoint (i.e., primary, secondary, or exploratory) supported by the assays utilizing PBMC as described below:

- If the PBMC are being used for **primary endpoint assays**, the protocol leadership may consider suspending enrollment of participants until the SPL returns to "Satisfactory" status in the subsequent quarterly testing cycle. In addition, the protocol leadership may decide to suspend the collection of blood for PBMC isolation from participants already enrolled. Another but less likely option would require the transfer of processing activities to another IVQAC Program-certified 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, specimen management, storage).
- 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, and the SPL returns to "Satisfactory" status with the IVQAC 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 blood collection for PBMC is halted, sites should not draw down funds for specimens not collected.

Until the issue is resolved, PBMC collected during the three months preceding the "Unsatisfactory" status will be considered for flagging purposes. Any flagging of PBMC will be proposed by the LOU to protocol chair(s) and sponsor. If a consensus for flagging is reached, the LOU will work with the protocol's statistical and data management organization (e.g., Emmes) to accomplish the flagging. Depending on conditions, flagged PBMC may be withheld from assays, or assay data may not be included in final study reports.

If the PBMC resubmission to the IVQAC Program in response to "Unsatisfactory" status is again problematic, the LOU will participate in the IVQAC call with the SPL and corresponding processor 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 PBMC collected during the

three months preceding the initial "Unsatisfactory" submission will be flagged in their respective study database until a "Satisfactory" submission is achieved. In rare occasions and at the discretion of the LOU and site PI, the LOU may request an on-site visit by an LOU representative to assist the SPL with improving their PBMC cryopreservation and handling techniques.

16.3.4.5. On Hold: An SPL may request that quarterly testing for PBMC cryopreservation be put "On Hold" due to operational circumstances (e.g., personnel shortage, change of instrument, no active protocols) for up to two quarters. The request must be submitted to the LOU via email at least one month prior to the submission date of the next quarter's PBMC for approval by DMID, the LOU and IDCRC Leadership. For requests beyond two quarters, sites will need to be re-approved by the IVQAC Program to resume participation. An SPL may not perform PBMC cryopreservation in any IDCRC protocol requiring viable PBMC, or serve as a backup SPL, while in an "On Hold" status. Note, a SPL may request to discontinue participation in the IVQAC Program (e.g., no upcoming active protocols, a shift in responsibilities, change in PI). The site will notify the LOU, IDCRC Leadership and other applicable stakeholders prior to informing the IVQAC Program.

16.3.4.6. Not Approved*:* If the SPL was unable or unwilling to improve performance after receiving notice that it was in "Unsatisfactory" status, it is no longer eligible to perform PBMC cryopreservation for IDCRC protocols.

16.3.5. Conditions for Approval & Other Measures

To maintain approval to cryopreserve PBMC for IDCRC protocols, each processor processing PBMC in an SPL must maintain a "Satisfactory" status with the IVQAC Program. Additionally, the SPL/processor(s) 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 leadership, DMID, and IDCRC Leadership on a case-by-case basis.

An SPL that is not approved because quarterly testing specimens were not submitted will be contacted by the LOU to discuss continued participation in the IVQAC Program.

If an SPL is not approved, the clinical site may identify a IVQAC Program-certified backup SPL with the capacity to cryopreserve PBMC for IDCRC protocol(s). The transfer of PBMC cryopreservation activities to the backup SPL 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.

16.4. 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, the IDCRC Leadership 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 Corrective and Preventive Action (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.

Version number	Approval Date DD MMM YYYY	Summary of Changes
1.0	14 NOV 2023	Initial Version
2.0	10 DEC 2025	• Modified language for better flow; clarified Duke and IDCRC LOU requirements for resolution of performance issues; updated terminology for consistency; clarified scoring criteria; added language to formalize communication. Added new program name (IVQAC). Updated with new IVQAC program description. Changed SPL/lab to technician-based program. Exchanged technician with the word processor. Added note about other entities oversight of the program. Added more detailed description of parameters considered for overall score. Deleted repeated information about enrollment into the program and actions required after IVQAC results are reported. Added note for SPLs that request discontinuation of the program. Simplified language for enrollment, approval and scorings steps. Deleted both graphs as they are not needed. Clarified that MOP supersedes the program descriptions and listed examples. Added more detailed description of scoring methos and IVQAC analysis. Minor grammar, language and formatting changes. Added language to cover discrepancies between program description and MOP. Added stakeholder approval. Added clarifying language for programs scoring methodology.