

Duke Virology Quality Assurance



Qualitative HIV-1 Total Nucleic Acid Testing (NAT) PT Program Participation Requirements and Scoring Procedures

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VQA Program Qualitative HIV-1 NAT Proficiency Testing Requirements

Introduction to Participation Requirements

The National Institute of Allergy and Infectious Diseases (NIAID), Division of AIDS/DAIDS (DAIDS) Virology Quality Assurance (VQA) Program provides quality assurance and proficiency testing to labs that are performing virologic-based assays for HIV and other pathogens in support of NIAID-funded clinical trials. All laboratories that intend to do qualitative HIV-1 total nucleic acid testing (NAT) for NIAID-funded clinical trials must have an approved status in the VQA Qualitative HIV-1 NAT proficiency testing program.

Participation in the real-time testing phase of the program can only occur after the successful testing of two five-member panels or completion of an assay validation. VQA Qualitative HIV-1 NAT proficiency panels (5 samples) are to be run every six months. The following sections detail the requirements to obtain and maintain approval to participate in qualitative NAT proficiency testing for NIH funded protocols.

Proficiency Testing Overview:

Laboratories participant in the Duke VQA Program are shipped proficiency panels twice per year. Each panel consists of 5 samples and will be named using the following format:

- NATYYYY_MMDER.01-05A-B,
 - NAT = PT program
 - YYYY = year of the PT
 - MM = month of PT testing
 - DER = specimen derivative (BLD, DBS, FWB, or PEL)
 - 01-05 = sample number
 - A-B = panel configuration

As a part of the program, the Duke VQA team will provide sites with a 5-member PT panel composed of HIV-positive and HIV-negative samples in whole blood (BLD), frozen whole blood (FWB), cell pellets (PEL), or dried blood spot (DBS) format to be assayed using qualitative HIV-1 nucleic acid assays. Each participating site will characterize the samples as HIV positive or HIV negative, which will be used to evaluate site performance.

Whole blood PT samples are collected by the VQA and shipped to sites within 24 hours of collection. If insufficient HIV-positive donors are available, HIV-negative blood will be collected and spiked with a targeted number of HIV-positive U1 cells. Sites must receive and process the samples within 10 days of collection. Proficiency in qualitative HIV-1 Nucleic Acid Testing is examined by each laboratory testing a panel of five coded specimens that consists of replicate samples from one or more blood donors. Each of the five samples should be extracted, amplified and detected according to their laboratory SOPs and the appropriate external controls must be included. The entire panel must be repeated if any of these criteria are not met.

Labs that participate in testing DBS samples, will receive a panel of 5 coded specimens (DBS Cards) that consists of single or replicate samples from one or more blood donors (spots on each card are from a single donor and contain 75ul of blood). A minimum of one spot from each of the five samples

must be extracted, amplified, and detected according to the laboratory's SOP used for HIV nucleic acid testing.

In addition to any kit controls required for qualitative testing, participating laboratories will also need to include a VQA extraction control with each PT run in order for the run to be considered valid. Sites will need to verify the extraction control results against the key provided by the VQA Program or through the use of the Laboratory Data Management System (LDMS) developed by Frontier Science. Extraction controls are a HIV-1 negative matrix aliquoted with or without the addition of a known number of HIV-1 positive cells from the U1 cell line. Extraction controls are provided in the following derivatives: frozen whole blood (0.125 mL aliquots of whole blood +/- U1 cells), PBMC suspension (0.1 mL aliquots of phosphate buffered saline with 20% fetal bovine serum containing 1 million PBMCs +/- U1 cells), and dried blood spots (0.75 mL of whole blood +/- U1 cells dried on a specimen card).

Proficiency scores are determined by comparing the outcome of the laboratory's assay results with the consensus infection status of the blood donors. Consensus is determined from results of the participating laboratories with 80% agreement required for each PT sample (see the **VQA Program Qualitative HIV-1 NAT Proficiency Testing Scoring Procedure** below). Data for the PT must also be valid, i.e. appropriate extraction controls were run in accordance with protocol testing requirements. Additionally, labs will be scored based on non-technical parameters. Penalties will be issued to lab if the data is late, labs do not respond to data queries from the Duke VQA, data containing PHI and/or PII is submitted to the VQA, or for data entry errors that are not corrected by the laboratory prior to the PT due date (please see **Non-Technical Performance** section below for additional details). All invalid proficiency panel samples and assays must be repeated; invalid assays or samples will result in penalty scores. A complete 5-member panel is needed and if a situation occurs where one or more of the samples is invalid, the VQA Program must be contacted for instructions on how to proceed. Laboratories will also be evaluated on transcriptional and computational errors; whenever possible, laboratories are encouraged to submit data using electronic files.

VQA Participation Requirements

New Laboratory Certification Testing

Participation in any VQA proficiency testing program must be approved by the VQA Contracting Officer Representative (COR, aka Project Officer). In order to be considered for participation in the VQA program, a new laboratory must be doing qualitative HIV-1 NA testing for a NIH-funded program. Once approved, the new laboratory will need to complete an application for participation in the program, which will outline the programs of interest and provide laboratory contact information. A new VQA laboratory number will be assigned to each VQA laboratory to uniquely identify that laboratory within the program.

Prior to enrollment into the proficiency testing program, all new laboratories must successfully pass two 5-member panels as prequalification; this may be done using stored frozen panels or may occur over successive whole blood round of regularly scheduled proficiency testing panels. Alternatively, the lab may complete a qualitative NAT validation assay. Once a lab successfully completes an assay validation or passes two five-member panels they will be provisionally approved for protocol testing. The frozen 5-member panels (DBS (0.075mL), cell pellets or whole blood (0.1mL)) are created at each round of proficiency testing for use in prequalification and are stored at -70°C to -90°C

A new laboratory must obtain an **APPROVED** performance rating prior to performing any protocol testing. Due to the nature of the real-time HIV NAT proficiency testing program, laboratories will achieve a **PROVISIONALLY APPROVED** performance rating once they pass the two 5-member member prequalification panels or perform a validation. However, this approval status is provisional, until a minimum of two real-time 5 member panels have been completed. If a laboratory obtains a score of C on two consecutive panels after prequalification, then they will be **APPROVED** for testing. If a laboratory obtains a score of C and PC on two consecutive panels after prequalification, then they must obtain a score of C on a third 5 member panel in order to become **APPROVED** for testing. If any other combination of scores are obtained on the first two to three 5 member panels after prequalification then the laboratory’s performance rating (PR) will be based on the sum of the panel scores from the four (4) most recent 5 member panels (Performance Score, PS). Individual panels are scored as a “1” for a Certified (C), “2” for a Provisionally Certified (PC), and “4” for a Probation (P). The corresponding panel scores and performance ratings are listed in **Table 1**(see the **VQA Program Qualitative HIV-1 NAT Proficiency Testing Scoring Procedure** section below for detailed information on scoring).

Table 1: VQA Performance Ratings

ASSESSMENT	PS	PR	Action
APPROVED	4-6	A	Eligible for protocol testing
PROVISIONALLY APPROVED	7-9	PA	Eligible for protocol testing at discretion of protocol virologist
NOT APPROVED	10-13	NA	Not eligible for protocol testing

Panels may be tested during regular rounds of proficiency testing, which occurs two times per year, or it may be Fast-Tracked using historical panels that have been pre-tested in the field. **NOTE: the normal track or fast track options must be begun within one year of passing the prequalification panel. If real-time testing is postponed beyond one year of testing, then the process of certification must be restarted.**

The final determination of whether or not a laboratory may perform protocol testing is at the discretion of the program leadership for that laboratory, not the Duke VQA.

New Assay Enrollment and Certification

A laboratory may request to:

- Obtain approval for protocol testing using multiple assays
- Switch platforms
- Certify additional instruments for VQA certified assays

All new assays and instruments enrolled in the VQA program need to be certified following the procedures outlined in the above section, **New Laboratory Certification Testing**. The VQA permits the use of two different assays; laboratories must obtain special approval from the VQA if they wish to become certified using more than two different assays. Each assay added to the VQA Program will be certified separately and the rules for achieving an **APPROVED** rating applies to all assays used within a laboratory.

The Duke VQA can assist in assay and instrument validations. Please contact the Duke VQA (vqa-dna@duke.edu) for more information about assay requirements or to request a validation plan and data submission template.

Maintaining Approval for Protocol Testing

To maintain an **APPROVED** certification status, a laboratory must participate in the routine proficiency testing program as scheduled by the VQA. Additionally, cumulative certification ratings based on the sum of the scores from the last four rounds of testing (see **Table 1** above) is used to determine the labs eligibility for protocol testing. Below is a list of PT scores and the associated point values:

- C (certified) = 1 point
- PC (provisionally certified) = 2 points
- P (probation) = 4 points

Additional points will be added if a laboratory received a non-technical penalty for data timeliness, data query responsiveness, or data submissions containing PHI and/or PII, or data entry errors (See **Non-Technical Performance** section below for additional details).

If a laboratory has a decline in status from approved to provisionally approved due to their cumulative score they would need to run and pass (with a score of C) two five-member panels before they can be fully approved for testing. Additionally, any score of “P” on a panel tested after qualification would require a laboratory to run and pass (with a score of “C”) two 5-member panels before full approval may be (re)achieved. The reapproval process may be fast-tracked using stored PT panels. If a laboratory receives a score of PC or P for a PT, they will receive an Investigation Report (IR) that is to be used to be reviewed and filled out by the laboratory. The IR form helps guide the lab in determining the root cause of the score for that PT.

If a lab is unable to participate in a PT testing round or submit data by the PT due date, the laboratory must contact the Duke VQA and request an exemption (not submit data for that round of testing), to be placed on hold, or extension. Duke VQA must approve all requests to be placed on hold or for exemptions and extensions. Only one exemption per four PTs can be granted to a participating site. Extensions may be granted for up to two weeks past the stated deadline for the PT. A laboratory may request an extension or exemption for reasons such as:

1. Panel stuck in customs
2. Delay of shipment
3. Panel was thawed upon receipt
4. Panel was lost during shipping
5. Reagents for analysis are backordered or delayed
6. Instrument was broken
7. Contamination of sample
8. Laboratory closures
9. Laboratory Accident (i.e. vials dropped and contents spilled)

A laboratory may request to be placed ‘on hold’ for up to 12 months without penalty (see section below on withdrawal from the program). If a lab is on hold for more than 12 months, they will need to be recertified as a new laboratory. Network approval must be obtained prior to resuming protocol testing.

Withdrawal/Removal

A laboratory may voluntarily withdraw from the VQA HIV RNA proficiency testing program at any time. A laboratory may request to be put “On Hold” as a result of operational circumstances (e.g., personnel problems, change of assay, etc.) for up to 12 months. After 12 months the laboratory will be automatically removed from that program. A laboratory that is “On Hold” for VQA proficiency testing must not test clinical trial specimens during the time in which they are “On Hold”. If a laboratory is “On Hold” for an extended period of time, they may be required to pass a proficiency testing panel or do extra testing before they are able to resume testing. This decision shall be made by the network leadership for whom the laboratory does testing.

Recertification

Labs Placed on Hold or Withdrawn from VQA Program

If a laboratory wishes to re-enter the VQA HIV RNA proficiency testing program subsequent to removal or placed on hold for longer than 12 months, that laboratory will need to be re-certified as a new laboratory (see section above).

Re-approval status may be attained after failure of a panel by obtaining two scores of “C” on two consecutive panels after the first failure. This may be achieved by running a “B” panel configuration for the panel that failed and running the next scheduled panel send-out (two 5-member panels must be completed and passed within 6 months of the first failure). Additionally, other archived panels may be tested if a laboratory wishes to recertify before the next scheduled panel send-out.

Following On-going Proficiency Testing Problems

A laboratory that is having ongoing NAT proficiency panel testing problems and is not maintaining an adequate approval certification status may be asked to undergo new laboratory re-certification (see section above).

Appeals

The VQA recommends scoring for proficiency panels based on the criteria defined for the program. The VQA Advisory Board (VQAAB) then reviews the scoring for each round of testing for labs that had a decline in status or had a problem that does not follow the VQA PT rules (the laboratory identities are blinded for this process). Any laboratory may appeal the score on a proficiency panel by submitting a letter or email to William Meyer III, Chair of the VQAAB (william.a.meyer@questdiagnostics.com). All appeals will be reviewed by the VQAAB to determine if a change in scoring is indicated. Laboratories will be notified of the outcome of all appeals

Practice Panels

Any laboratory may obtain retrospective HIV RNA proficiency panels prior to enrollment in the program, or at any time during their participation in the VQA program. Once a panel has been tested and analyzed as a ‘practice’ panel, it cannot be reclassified for use in laboratory certification. Results from these ‘practice’ panels will be assessed and results returned to the laboratory, but no certification score will be assigned.

Change in Status Letters

A laboratory will receive a change in status letter if they obtain a score on a round of testing that changes their overall performance rating. This letter will document the laboratory’s scores over the last four rounds of testing and will indicate when a change in status (performance rating) has

resulted. A copy of this letter will be sent to the director of the laboratory and the network laboratory group for whom the laboratory does testing, as appropriate. Letters will be sent to notify individuals of both negative and positive changes in approval ratings. The VQA submits the letters on behalf of the VQAAB but has no control over the implementation of rules governing the ability of a site to continue protocol testing. All questions surrounding a laboratory’s ability to resume or discontinue protocol testing should be directed to the respective network laboratory group or leadership.

VQA Program Qualitative HIV-1 NAT Proficiency Testing Scoring Procedure

Introduction to PT Scoring

Proficiency scores are determined by comparing the outcome of the laboratory’s assay results with the consensus infection status of the blood donors. Consensus is determined from results of the participating laboratories with 80% agreement required for each PT sample. Each site receives a score based on the criteria listed in **Table 2** and the scores are tracked over time. The cumulative table will track laboratories proficiency testing scores, and will indicate when a recertification panel has been performed by placing an asterisk (*) after the score received on the first 5-member panel that follows the recertification panel. Scores for the recertification panel will not be tracked on the VQA cumulative table.

Scoring Criteria for VQA Quantitative HIV-1 RNA Proficiency Testing

Technical Performance

Table 2 summarizes how labs are scored. Briefly, a laboratory will receive a score of C if their run is valid and all of their results match the consensus for each sample. If a lab has a minor problem such as one false negative or a single invalid result due to a failed internal instrument check they will receive a score of PC. A score of P will be given to any lab that has major issues such as an invalid run, two or more false negative results, one false positive result or two or more invalid results per round.

Each laboratory must have a valid test run to receive a passing score. A run is considered valid if VQA extraction controls are run and verified by the lab. Verification and running controls should be completed prior to submission of PT data.

Table 2: Description of criteria for each possible score

Score	Criteria
C	<ul style="list-style-type: none"> • Run is valid (lab ran appropriate controls) • Results match the consensus
PC	<ul style="list-style-type: none"> • Run is valid (lab ran appropriate controls) • Single false negative result or invalid sample (sample failed internal instrument checks)
P	<ul style="list-style-type: none"> • Run is invalid, two or more false negatives, or 1 or more false positives

To avoid penalties due to submission of invalid sample results or an invalid run, the VQA recommends that all labs repeat testing prior to submission of data. Laboratories should have enough samples to repeat testing if needed. However, if your laboratory does not have sufficient samples to repeat testing, contact the VQA via vqa-dna@duke.edu for a replacement instructions or sample/panel.

Non-Technical Performance

A laboratory will receive a non-technical penalties summarized in **Table 3** and described below.

Table 3. Non-technical Penalties and Impact on PT Score

Score	Points	Penalty Type	Description of Penalty
C1 ^T	2	Data Timeliness	Data submitted late without requesting an extension
PC1 ^T	4		
P1 ^T	4		
C1 ^Q	2	Data Query Responsiveness	Not responding to queries about data within one week (5 working days)
PC1 ^Q	4		
P1 ^Q	4		
C1 ^P	2	Data Submission Containing PHI and/or PII	Laboratory submitted data that contained PHI and/or PII
PC1 ^P	4		
P1 ^P	4		
C1 ^D	2	Data entry errors	Laboratory submitted data with entry errors that were not caught and corrected prior to the PT due date
PC1 ^D	4		
P1 ^D	4		

A laboratory may receive penalties for the following items:

1. **Data Timeliness**

A due date is assigned to each 5-member panel. Data must be submitted via the Duke VQA Web Application by the deadline or data will receive a penalty. A laboratory may request an exemption, extension, or to be placed on hold by emailing vqa-dna@duke.edu. If no contact is made prior to the due date, and the data are received late once within four rounds of testing, then the proficiency testing score will be down-graded one level (i.e. a technical score of C would be assigned a score of C1^T, which equates to 2 points for a panel score). If data are received late for the second time in four rounds of testing, then the proficiency testing score will be down-graded two levels (i.e. a technical score of C would be assigned a score of C2^T, which equates to 4 points for a panel score) summarized in **Table 3**.

2. Data Query Responsiveness

Occasionally, a query is sent to a laboratory to clarify or fix a problem noted in their VQA submission. The Duke VQA will email the testing laboratory and ask them to follow up on the query. If the laboratory resolves the query within a week of receiving the original query (5 working days, excluding any holidays), then no penalty will be assessed. If no resolution is received, a second query will be sent to the testing laboratory and will include affiliated network laboratory coordination center. If the query is not resolved within one week (5 working days excluding holidays) of the second query, then the data will be scored as a minor late query response. A third query will be sent to the testing laboratory, including the VQA manager, and the affiliated network laboratory coordination group. If the problem is still not resolved within one week (5 business days excluding holidays) then a major problem will be assessed. If a minor late query resolution is noted, the proficiency testing score will be down-graded one level (i.e. a technical score of C would be assigned a score of C1^Q, which equates to 2 points in a panel score; a technical score of PC would be assigned a score of PC1^Q which equates to 4 points in panel score). If a major late query resolution is noted, then the proficiency testing score will be down-graded two levels (i.e. a technical score of C would be assigned a score of C2^Q, which equates to 4 points for a panel score). A major late query resolution will receive the maximum of 4 points for a panel score.

3. Data Submissions Containing PHI and/or PII

PHI (protected health information), PII (personally identifiable information), or any information that could link submitted data to an individual participant must not be included in any data files submitted to the VQA for analysis.

Note: it is at the discretion of the VQA to reject data sets that are presumed to contain PHI/PII.

If, during QA of the data, the **VQA** finds PHI or PII, a penalty will be assessed. All data that contains PII or PHI will be expunged from the database and laboratories will be required to remove the information and resubmit their data. Data resubmitted after the due date, without an exemption request, will also receive a late penalty score.

If a data set contains PHI/PII, the proficiency testing score will be downgraded one level for a first offense (i.e. a technical score of C would be assigned a score of C1^P for the first incident, which equates to 2 points for a panel score). For subsequent offenses within 4 rounds of testing, the proficiency testing score will be downgraded an additional level (i.e. a technical score of C would be assigned a score of C2^P, which equates to 4 points for a panel score). Laboratory directors, network coordination centers and the Division of AIDS will be notified if a laboratory submits data containing PHI/PII.

Note: Any combination of penalties (technical or non-technical) will result in a maximum panel score of 4 points (e.g. C1^{Q,P}, PC1^T, etc).

4. Data Entry Errors

Data for each Qualitative HIV-1 NAT PT is submitted using the VQA web-system, which requires entry of whether the sample is positive or negative for HIV-1 and upload of raw data files. Participating sites have the ability to save their data in the web-system and review it before submission. Prior to the PT due date, a site may request to re-open their PT to correct data entry errors. However, sites will not be able to correct any data entry errors after the PT due date. Data that is submitted to the VQA that contains data entry errors such as entering the wrong values, entering data in the wrong location, switching samples, or submitting the wrong file could result in a non-technical penalty.

If a data entry error occurs once within four rounds of testing, the proficiency testing score will be down-graded one level (i.e. a technical score of C would be assigned a score of C1^D, which equates to 2 points for a panel score). If a data entry error occurs for the second time in four rounds of testing, then the proficiency testing score will be down-graded two levels (i.e. a technical score of C would be assigned a score of C2^D, which equates to 4 points for a panel score) as summarized in **Table 3**.