

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 proficiency testing (PT) 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 Acceptable 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 completion of an assay validation and testing of two five-member panels. 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:

Beginning January 2023 new scoring rules will be in effect (please see **VQA Program Qualitative HIV-1 Proficiency Testing Scoring Procedure** below for detailed information on scoring procedures). Under the newly approved scoring system sites will now receive a score of either Satisfactory or Unsatisfactory for datasets submitted for each PT. Additionally, there will be two performance rating categories, Acceptable or Not Acceptable. A laboratory must have a Satisfactory grade for 2 out of the 3 past PTs to have an Acceptable performance rating.

The performance ratings are listed in **Table 1** (see the **VQA Program Qualitative HIV-1 Proficiency Testing Scoring Procedure** section below for detailed information on scoring):

Table 1: VQA Performance Ratings

Assessment	PR	PT Score
Acceptable	A	SSS, SUS, USS, SSU
Not Acceptable	NA	UUU, SUU, USU, UUS

PR: Performance Rating
 A: Acceptable; NA: Not Acceptable
 S: Satisfactory; U: Unsatisfactory

Laboratories participating 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 prepared by the VQA and shipped to sites. If insufficient HIV-positive donors are available, HIV-negative blood will be collected and spiked with a targeted number of HIV-positive U1 cells. Qualitative HIV-1 Nucleic Acid PT involves 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 standard operation procedures (SOPs). PT panel samples must be repeated if any manufacturer or laboratory-defined validity criteria are not met. Invalid assays or samples will result in penalty scores.

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 75 μ L 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. PT panel samples must be repeated if any manufacturer or laboratory-defined validity criteria are not met. Invalid assays or samples will result in penalty scores.

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). Transcriptional and computational errors will result in a score of Unsatisfactory; whenever possible, laboratories are encouraged to submit the raw data file.

VQA Participation Requirements

New Laboratory Qualification 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 planning to, or currently performing qualitative HIV-1 nucleic acid testing for a NIH-funded program. Once approved by the VQA COR, 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 participant laboratory is identified by a Harmonic ID (HID), Laboratory Data Management System (LDMS), or VQA-assigned number.

A new laboratory must obtain an **Acceptable** performance rating prior to performing any protocol testing. In order to achieve an Acceptable performance rating for the Qualitative HIV-1 NAT PT program, a laboratory must perform an assay validation (VQA-provided or in-house) and pass two consecutive or two of the most recent three 5-member panels. The testing may be done during normal track testing (panels will be received every six months), or fast-tracked using historical panels. In order to identify problems early in the process, the first 5-member panel must be

completed and scored prior to testing the next set of samples. If proficiency testing is postponed beyond one year following the qualification process, then another two 5-member panels must be passed prior to participation in the VQA Qualitative HIV-1 NAT PT program.

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

New Assay Enrollment and Qualification

A laboratory may request to:

- Qualify additional assays for protocol testing
- Switch platforms
- Validate additional instruments for qualified assays

All new assays and instruments enrolled in the VQA program need to be qualified following the procedures outlined in the above section, **New Laboratory Qualification Testing**. The VQA permits the use of two different assays; laboratories must obtain special approval from the VQA if they wish to become qualified using three or more different assays. Each assay added to the VQA Program will be qualified separately and the rules for achieving an Acceptable performance rating applies to all assays used within a laboratory. If a laboratory uses a manual extraction method as a backup for their automated extraction instrument, then the laboratory will need to demonstrate ongoing proficiency testing using both extraction methods.

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

Maintaining Acceptable Performance Rating

To maintain an **Acceptable performance rating** a laboratory must do the following:

- Participate in the routine proficiency testing program as scheduled by the VQA
- A laboratory must have a Satisfactory score for 2 out of the 3 most recent rounds of proficiency testing.

Extensions will no longer be granted to sites. PT due dates will be adjusted as needed if the shipment of a panel is delayed. If a lab does not submit data by the PT due date the lab will receive a score of Unsatisfactory. However, a laboratory may request a PT exemption if they are unable to participate in proficiency testing due to circumstances out of their control. All exemption requests must be approved by the VQA. Acceptable reasons to temporarily be exempt from proficiency testing include:

1. Force Majeure (hurricane, tornado, flood, fire, etc.)
2. Laboratory closures for emergency circumstances (fire, pandemic, radiation leak, flood, electric issues, etc.)
3. Supply chain issues with vendor
4. Broken instrument
5. Government shutdown or political unrest

Exemption requests should be made to vqa-dna@duke.edu prior to the start and/or close of the PT. A laboratory may receive a score of Unsatisfactory if the reason for not submitting results does not meet one of the acceptable criteria for exemption. **A laboratory must contact their network leadership if they are exempted from a round of VQA Qualitative HIV-1 NAT proficiency testing.**

Withdrawal/Removal

A laboratory may voluntarily withdraw from the VQA Qualitative HIV-1 NAT proficiency testing program at any time. A laboratory that has not participated in the VQA Qualitative HIV-1 NAT proficiency testing program for more than 12 consecutive months will automatically be removed from the program.

Requalification

A laboratory will have to requalify if:

- A laboratory wishes to re-enter the Qualitative HIV-1 NAT proficiency testing program after removal or has not participated in longer than 12 months;
- The laboratory has ongoing problems with proficiency panels

The laboratory will need a Satisfactory score on two (2) consecutive or 2 of the most recent 3 five-member panels. Archived panels may be tested if a laboratory wishes to requalify before the next scheduled panel send-out.

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 Qualitative HIV-1 NAT 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 proficiency 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.

Scoring Criteria for VQA Qualitative HIV-1 NAT Proficiency Testing

Technical Performance

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

Table 2: Description of criteria for each possible score

NAT Criteria	Score
<ul style="list-style-type: none"> • Run is valid (lab ran appropriate controls) • Results match the consensus 	Satisfactory (S)
<ul style="list-style-type: none"> • Single invalid sample (sample failed internal instrument checks) 	Satisfactory (S) +PIA
<ul style="list-style-type: none"> • One false positive or false negative 	Unsatisfactory (U)
<ul style="list-style-type: none"> • Two or more invalid results per round 	Unsatisfactory (U)
<ul style="list-style-type: none"> • Data not submitted or submitted after the PT due date 	Unsatisfactory (U)

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 or encounters repeated failures with a PT panel member, contact the VQA via vqa-dna@duke.edu for a replacement sample/panel.

Data Entry Errors

Data for each Qualitative HIV-1 NAT PT is submitted using the VQA web-system, which requires entering the HIV-1 status of the sample (positive or negative) and allows upload of the raw data files. Participating sites have the ability to save their data in the web-system and review it to correct data entry errors before submission. However, sites will not be able to correct any data entry errors after submission. Data that are submitted to the VQA that contains data entry errors such as entering the wrong values, entering data in the wrong location, or switching samples may result in a score of Unsatisfactory.