VQA PROGRAM TESTING REQUIREMENTS TO OBTAIN APPROVAL TO PARTICIPATE IN RNA PROFICIENCY TESTING FOR NIH-FUNDED PROTOCOLS

Pre-certification Testing: 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.

New Laboratory Certification Testing: Prior to enrollment into the real-time HIV RNA proficiency testing program, all new laboratories will need to test a 20-member prequalification panel containing coded plasma specimens. The results from this panel will be submitted for scoring either via the LDMS or via the DSS web utility. Laboratories will need to register with FSTRF (http://www.fstrf.org) in order to gain access to the VQA web utilities. A score of 'C' on the prequalification panel is required to enter into the HIV RNA proficiency testing program. A score of a 'PC or a P' will require the laboratory to run another 20-member panel prior to advancing. A second score of a 'PC or a P' will require intervention by the *VQA LAB* prior to any additional testing. *NOTE: the normal track or fast track options must be begun within one year of passing the certification panel. If real-time testing is postponed for 6-12 months after certification, the VQA recommends that one 5-member panel be completed and scored prior to the initiation of protocol testing. If real-time testing postponed beyond one year of testing, then another 20-member prequalification panel must be passed before participation in the VQA HIV RNA proficiency testing program may occur.*

Normal Track and Fast Track Approvals: A new laboratory must obtain an **APPROVED** performance rating prior to performing any protocol testing. Due to the nature of the real-time HIV RNA proficiency testing program, laboratories will achieve a **PROVISIONALLY APPROVED** performance rating once they pass the 20 member prequalification panel. 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 fast-track **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 the table below:

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

NOTE: 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 VQA Laboratory.

Assays: A laboratory may request to obtain approval for multiple assays. 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 will be certified separately and the rules for achieving an **APPROVED** rating described above will apply 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. Please contact the VQA manager (vqa@rush.edu) for additional information about assay requirements.

Continued Approval: To maintain an **APPROVED** certification status, a laboratory must participate in the routine proficiency testing program as scheduled by the ***VQA LAB*** and maintain a PS of 4-6 points. The ***VQA LAB*** manager or director must approve all requests for exemptions or extensions. A laboratory may request one extension per year. A laboratory may request to be placed 'on hold' for up to 12 months without penalty (see section below on withdrawal from the program). Laboratories that are placed on hold for more than 6 months will be expected to run and pass the two most recent 5-member panels upon re-entering the program. Network approval must be obtained prior to resumption of protocol testing.

Withdrawal/Removal: A laboratory may voluntarily withdraw from the VQA HIV RNA proficiency testing program at any time. A laboratory may also request to be placed 'ON HOLD' as a result of operational circumstances (e.g. personnel problems, laboratory issues, etc.) for up to 12 months. A laboratory may not perform protocol testing while the laboratory is 'ON HOLD'. A laboratory that has not participated in the VQA HIV RNA proficiency testing program for more than 12 consecutive months will automatically be removed from the program.

Re-certification: If a laboratory wishes to re-enter the VQA HIV RNA proficiency testing program subsequent to removal, that laboratory will need to be re-certified as a new laboratory (see section above). A laboratory that is having ongoing RNA proficiency panel testing problems and is not maintaining an adequate approval certification status may be asked to undergo new laboratory re-certification.

Proficiency Testing:

Laboratories will be shipped a 20-member panel every eight months. Each panel consists of 5 samples (01-05, 06-10, 11-15, and 16-20). Every two months, laboratories will test the 5-member panel in a single assay. Any 5-member panels that are repeated due to technical problems on the previous round must be assayed separately from any other VQA HIV RNA proficiency testing samples. Scores will be provided after each round of testing. Each analysis will include a minimum of 20 samples (data from the last four 5-member panels). Evaluating 20 samples at every round provides sufficient statistical power for these longitudinal analyses. Cumulative certification ratings will be based on the sum of the scores from the last four rounds of testing (see Table 1 below).

Scores for each round of testing will be derived from an analysis of precision, accuracy, specificity, sensitivity, and assay validity. Precision assessments will be based on the combination of inter-assay and intra-assay variations to better monitor the variability associated with real-time testing. Expected values for total variation were derived empirically from VQA proficiency testing data, and the distribution of total assay variation was determined using Monte Carlo simulations. Proficiency testing data will be scored for deviations in total assay variability based on these cutpoints. Comparing the average $\log_{10}$ recoveries in a laboratory with the median $\log_{10}$ recovery across all laboratories using the same test will assess accuracy. Median values will be used instead of mean values to eliminate effects of outliers. Sensitivity will be determined for each assay by testing specimens at or near the limit of detection. The bioMerieux NucliSens EasyQ assay (assuming a 1mL sample input) and the Versant bDNA assay are challenged for sensitivity using samples with a nominal value of 200 copies/mL; the Abbott RealTime HIV-1 Assay and the Roche COBAS AmpliPrep/COBAS TaqMan, v2 assay are challenged for sensitivity using samples with a nominal value of 50 copies/mL. Rates of false positive results (specificity) will be ascertained using blinded samples that do not contain HIV.

Assay/specimen validity will be determined at each round of testing, based on the Real-Time HIV RNA Validation Criteria posted on the VQA website (https://www.hanc.info/labs/labresources/vqaResources/ptProgram/Pages/default.aspx). All invalid proficiency panel samples and assays must be repeated; invalid assays or samples will result in penalty scores. Laboratories will also be evaluated on transcriptional and computational errors; whenever possible, laboratories are encouraged to submit data using electronic files. Laboratories that do not use the LDMS to run proficiency samples may submit files that are captured electronically from the assay instrument or will need to submit data on the EXCEL spreadsheets provided by the ***VQA LAB***.

Protocol Testing Eligibility Based on Proficiency Testing Cumulative Scores: A laboratory's performance rating will be based on the sum of the scores from the latest four (4) rounds of proficiency testing (cumulative rating). Scores from each round of testing will be added: '1' for a 'C' (certified), '2' for a 'PC' (provisionally certified), and '4' for a 'P' (probation). Since each round of proficiency scoring will encompass data from four panels (20 samples), a laboratory will be expected to repeat a problematic 5 member panel by the next round of testing (in addition to the new 5 member panel). The repeated data will replace the problematic data and will be used in determining the proficiency score for the next round of testing – a separate analysis of repeat data will NOT be performed. Laboratories will only be able to repeat samples from the previous round, so it is extremely important that the analysis from each round is reviewed carefully to note when problems may start to occur. If a laboratory opts not to repeat samples, then problems that were noted may carry over to subsequent analyses. As necessary, the ***VQA LAB*** will ship an extra 5-member panel to a laboratory that fails a round of testing. The testing laboratory will need to run the new panel and submit the data to the VQA for analysis. Both panels will need to be completed in the designated time frame, but should not be run within the same assay. Repeat testing of a given 5-member panel will only be permitted once. Ongoing scoring problems may require a laboratory to undergo re-certification (successfully pass a 20-member panel) in order to maintain eligibility for protocol testing.

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 (the laboratory identities are blinded for this process) and approves or changes the scores provided. Once approved, the scores are released to the laboratory. Any laboratory may appeal the score on a proficiency panel by submitting a letter or email to William Meyer III, Chair of the VQAAB (meyerb@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.

Change in Status Letters: Subsequent to review of the VQA score recommendations by the VQAAB, the VQA will generate a "Change In Status Letter" if a laboratory obtains 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.