VQA Proficiency Testing Scoring Document for Qualitative HIV-1 DNA PCR

The VQA HIV DNA PCR Proficiency Testing Program has two phases: a pre-qualification phase and a real-time proficiency testing phase. The pre-qualification phase involves running a panel of 30 PBMC pellet specimens that are spiked with 0, 2, 5, 10 or 20 copies of HIV proviral DNA per amplification input. The 8E5/LAV cell line is used to seed HIV seronegative human PBMCs with a known concentration of HIV proviral DNA copies. The input number per amplification is determined based on the assumption that 1/4th of the extracted pellet will be put into amplification. For example, in the Roche Amplicor HIV-1 DNA Test, the pellet is extracted in 200uL of buffer and 50uL of the extract is amplified in the assay. As a result, each pellet actually contains 4X the reported copy number (e.g. the 20copy pellet actually contains 80 copies of proviral HIV DNA per pellet).

The purpose of the pre-qualification phase is to determine if a laboratory is able to correctly perform the HIV DNA assay. Sample preparation is not evaluated during this phase of testing. Pre-qualification panels are evaluated for assay validity and panel results. Assay validity is based on the results obtained on VQA DNA Copy controls and VQA real-time blinded controls that are included in the testing. The expected results for the panel are based on the configuration and follow the same criteria used to evaluate HIV DNA copy controls, with the addition of a 2 copy control, which is monitored for assay sensitivity. The scoring criteria that are described here are based on results from early proficiency panels and discussions with ACTG virologists regarding the relative importance of false positive and false negative results. The scoring criteria for the VQA HIV DNA pre-qualification panel are as follows:

Performance criteria are based on the following conditions:

1. A run must be valid - all controls must satisfy the criteria defined in the DNA SOP (see p.3-4 of this document)
2. Pellets with a nominal concentration of 0 copies must be negative (OD < 0.2)
3. Pellets with nominal concentrations of 10 and 20 copies must be positive
4. Pellets with nominal concentrations of 20 copies must yield an OD greater than 2.0

Each of these criteria must be satisfied in order to successfully pass this panel. A table of the laboratory's results, along with the nominal concentration for each sample, is provided in a report. A statement of the validity of the run based on the results for the blinded controls is also provided.

In addition, the data may be reviewed for assay sensitivity. The expectation for sensitivity is as follows:

1. Pellets with a nominal concentration of 2 copies will be positive at least 50% of the time, and
2. Pellets with a nominal concentration of 5 copies will be positive at least 95% of the time.

While this criterion is not part of the certification, it is provided to the laboratory for evaluation of their performance.

If the run is valid, then the score is based on the results from the panel members that have a nominal concentration of 0, 10 or 20 HIV-1 proviral DNA copies. A score of PC is assigned if the results include one false negative or one indeterminate outcome. A score of P is assigned

for one false positive, at least two false negatives, at least two indeterminate outcomes or a combination of at least one false negative and one indeterminate result. A score of C is assigned only if the run is valid and results (positive or negative and OD criteria) are correct on all samples.

The real-time proficiency testing phase of the VQA HIV DNA program involves the running of a panel of 8 coded whole blood samples. The purpose of this phase of testing is to fully evaluate a laboratory's ability to receive, process and analyze whole blood samples for the presence of HIV proviral DNA. The VQA laboratory evaluated the stability of HIV DNA in whole blood and determined that the whole blood may be used up to 10 days after collection for the detection of HIV DNA, as long as the samples are maintained under refrigerated conditions (J Clin Microbiol. 2005 Aug;43(8):4249-50). This enables the VQA to evaluate all laboratories in the same manner without constraints that may be imposed due to shipping delays.

The configuration of each whole blood panel varies and includes whole blood which is obtained from both HIV-positive donors and HIV-negative donors. The scoring criteria for the real-time proficiency testing VQA HIV DNA panel are as follows:

Performance criteria are based on the following conditions:

1. A run must be valid - all controls must satisfy the criteria defined in the DNA SOP (see p.3-4 of this document)
2. The results for each panel member reported by the laboratory must match the key provided by the VQA laboratory.

If the run is valid, then the score is based on the results from the eight samples. A score of PC is assigned if the results include one false negative or one indeterminate outcome. A score of P is assigned for one false positive, at least two false negatives, at least two indeterminate outcomes or a combination of at least one false negative and one indeterminate result. A score of C is assigned only if the run is valid and results (positive or negative) are correct on all samples.

VQA DNA SOP, v3.0, 9-21-2005

VQA Reagent Setup – Roche Amplicor HIV-1 Test, v1.5

	1	2	3	4	5	6	7	8	9	10	11	12
A	**0 copy**	sample 1	sample 5	sample 9	sample 12	sample 16	sample 20	sample 24	sample 28	sample 31	sample 35	sample 39
B	**0 copy**	sample 1	sample 5	sample 9	sample 13	sample 17	**blinded #4**	sample 24	sample 28	sample 32	sample 36	sample 40
C	**5 copy**	sample 2	sample 6	sample 10	sample 13	sample 17	sample 21	sample 25	sample 29	sample 32	sample 36	sample 40
D	**5 copy**	sample 2	sample 6	sample 10	sample 14	sample 18	sample 21	sample 25	sample 29	sample 33	sample 37	**blinded #6**
E	**10 copy**	sample 3	sample 7	**blinded #3**	sample 14	sample 18	sample 22	sample 26	sample 30	sample 33	sample 37	sample 41
F	**20 copy**	sample 3	sample 7	sample 11	sample 15	sample 19	sample 22	sample 26	sample 30	sample 34	sample 38	sample 41
G	**blinded #1**	sample 4	sample 8	sample 11	sample 15	sample 19	sample 23	sample 27	**blinded #5**	sample 34	sample 38	sample 42
H	**blinded #2**	sample 4	sample 8	sample 12	sample 16	sample 20	sample 23	sample 27	sample 31	sample 35	sample 39	sample 42

Roche Qualitative DNA Assay Setup:

The VQA provides copy controls and real-time blinded pellets for use in the Roche Amplicor HIV-1 DNA PCR Test, v1.5. The copy controls provided include the following:

VQA Control name	Estimated concentration (copies DNA)	Replicates
0 copy control	0	duplicate
5 copy control	5	duplicate
10 copy control	10	single
20 copy control	20	single

Each of these copy controls must be extracted in singly, but amplified and detected in the number of replicates listed above. Additionally, there are criteria that must be satisfied for each of these controls to be valid:

1. The 0 copy VQA control must be Non-reactive (<0.2)
2. The 10 copy control must have an OD >0.8.
3. The 20 copy VQA control must have an OD >2.000.

Real-time blinded pellets must also be included in each of the runs. Two blinded pellets must be run, each in singleton. Two pellets must be included for the first ten samples (run in duplicate) or twenty samples (run in singleton). An additional blinded pellet must be added after every 10 (duplicate amplifications) or 20 (single amplifications) specimens. The results for these blinded pellets must be verified in order to determine if an assay is valid. If the LDMS (Laboratory Data Management System) is used, the program will automatically verify the results and determine if the assay is valid. If the LDMS is not used, the user may email the results for the blinded pellets to dna.pcr.pellet@fstrf.org. Once the results have been verified, and if the controls are valid, then the run may be considered valid.

If a sample needs to be re-extracted and/or reamplified and detected, then a new set of controls must also be processed in the same manner. New DNA copy controls and new blinded pellets must be included for any new re-extraction. If a sample only needs to be re-detected, then new control results do not need to be provided. An invalid control result can only be re-detected in order to validate a run. If a re-extraction and/or re-amplification are needed then the entire run should be repeated.

The following template may be used when emailing results to the DNA PCR logon for validation:

Dear FSTRF Staff,

Please verify the following results of blinded pellet IDs for the Roche Amplicor HIV-1 DNA PCR Test, v1.5.

The results are as follows:
Lab Number: 000
Requested by: Technician Name
Method of Assay: Roche Amplicor HIV DNA, v1.5
Date of Request: 9/21/05
Method of Request: Email

Blinded Pellet Number	Laboratory Result
022d.019	3.25, Reactive
022d.020	2.055, Reactive
022d.021	0.068, Non-reactive

In addition to your blinded pellets, the results for our DNA copy controls are as follows:

DNA Copy Control ID	Laboratory Result
0 copy	0.075, 0.065, Non-reactive
5 copy	1.568, 2.038, Reactive
10 copy	3.999, Reactive
20 copy	3.999, Reactive

Please email these results to DNA.PCR.PELLET@FSTRF.ORG for validation.