

VQA HIV RNA Proficiency Testing

HIV RNA – New Lab

- pass 20 member panel with a score of C
- Scored on intra-assay precision, sensitivity, specificity, and accuracy
- Must score a C on 2 real time 5 member panels (or receive 2 C's and 1 PC) to be approved for protocol testing.

HIV RNA - Problems

- During 5 member testing, problems may arise that may affect future analysis.
- Lab is given option to repeat the 5 member “B” panel when they score C or PC.
- Lab must repeat the 5 member panel when they score a P.
- The VQA will contact a lab if a score of PC or P was received. The Lab must contact the VQA if they opt to repeat a panel that received a score of C.

HIV RNA – “B” Panels

- “B”-Panels can only be repeated and submitted with the next round.
- “B” Panels can only be repeated one time only.
- “B” Panels need to be assayed separately from any other VQA Panel.
- “B” Panel data and new “A” Panel data are due by the assigned deadline for the next round of testing.

HIV RNA - Analyses

- Each VQA analysis must include a minimum of 20 samples
- Certifying panel data are included in real-time testing analyses for 3 rounds testing- the data are dropped when 4 five member panels are available for evaluation.
- After each new round, a new data set is added and the oldest data set is removed from the analysis.
- “B” Panel data are not analyzed separately – they simply replace problematic “A” data.

HIV RNA – When to Repeat

- Need to weigh the odds – repeating may cause more problems
 - Identify the problem (e.g. precision, invalids, false negatives, etc.)
 - Determine if the problem occurred in the most recent five member panel
 - Determine if the repeat will ‘fix’ the problem
 - Contact the VQA if you don’t understand the problem

HIV RNA – When to Recertify

- Sometimes you may be better off, recertifying
 - Is the problem associated with multiple data sets (i.e. shifts or trends)
 - Did the error occur on a “B” Panel that cannot be repeated
 - Make sure the problem is not kit related – this may only temporarily ‘fix’ problems or create future problems (e.g. precision, accuracy)

HIV RNA – Review Your Analysis

- Always ask about your panel results if you are not clear what the problem was.
- Double check your results with what NERI summarizes to make sure there were no data errors.
- Most importantly use the LDMS so no data transcription errors occur on your end.

HIV RNA cumulative scoring

- Cumulative score is obtained by adding the numeric panel score for the last 4 rounds
 - $C=1$
 - $PC = 2$
 - $P = 4$
- There are late penalties as well
 - $C1 = 2; C2 = 4$
 - $PC1$ and $PC2 = 4$
 - $P1$ and $P2 = 4$

HIV RNA - Ratings

- Cumulative Scores (CS) and Ratings (R)
 - CS of <6 = Approved
 - CS of 7 – 9 = Provisionally Approved
 - CS of >10 not approved
- HPTN
 - Panel score = P; **STOP** testing until Panel score = PC or C

HIV RNA – Precision Stats

- Log10 recovery is assessed for every round
- The variance is added between rounds and within rounds to derive the total assay SD
- Lab recovery is compared against the nominal or expected value for each specimen
- Target log10 SD = 0.15
- Penalties assess if a lab's SD is significantly different than 0.15 (usu. SD ~ >0.22 = PC, SD ~ >0.24 = P)
- Sample log10 recovery STATS for VQA data:

LAB	kit	round	n	min	mean	max	var_ round	var_ error	total sd
78	RS	10	12	-0.182	-0.020	0.094			
78	RS	11-2	3	-0.034	0.006	0.058			
78	RS	12-1	3	0.009	0.038	0.059			
78	RS	12-2	3	-0.117	0.084	0.243	0.001	0.008	0.092

HIV RNA - Accuracy

- Accuracy is assessed by comparing the average log₁₀ recovery in a lab to the expected
- The expected log₁₀ recovery is defined as the median log₁₀ recovery across all laboratories using the same kit
- The accuracy statistic is defined as:
 - $(R-M)/S$ where R is average log₁₀ recovery in a laboratory, M is median log₁₀ recovery across all laboratories and S is the standard error of the mean.
- An accuracy statistic of >3 or $<-3 = PC$; and accuracy statistic of >4 or $<-4 = P$

HIV RNA - Sensitivity

- Sensitivity is assessed by determining the number of negative results obtained on samples with nominal values at the 95% detection rate for that assay
- 95% cutoff values are determined by kit:
NucliSens = 250cp/mL, Standard Roche Monitor = 500cp/mL, UltraSensitive Roche = 50cp/mL, bDNA = 75cp/mL
- Typically 3-5 samples are included in this analysis
 - 1 false negative is typically no penalty
 - 2 false negatives are typically a score of PC
 - More than 2 false negatives are typically a score of P

HIV RNA - Specificity

- Specificity is assessed by monitoring the results obtained on specimens that do not contain HIV
- HIV-seronegative plasma used to create the panel is used to assess specificity
- All samples are expected to be undetectable, NO HIV is present
- 1 false positive result = PC; 2 or more false positive results = P

HIV RNA – Assay Validity

- Assay/specimen validity is assessed based on the manufacturer's guidelines for a valid assay
 - Valid QS, valid controls, out-of-sequence errors
- Additional criteria may be added as defined by the VQA laboratory
 - OD Ratio errors, transcriptional errors
- NERI will recalculate every reported estimated using the raw data provided by the laboratory
- Tools in the LDMS or VQA spreadsheets are available to assess real-time validation checks
- 1 invalid results = PC; 2 or more invalid results = P
- Invalid results will carryover and affect future analyses

HIV RNA – Data Timeliness

- Data must be received by the assigned due date or a penalty will be assessed
 - A lab may request an extension, without penalty, if they contact the VQA prior to the assay deadline
 - Make sure a new deadline is clearly defined for each assay being used
- “Late Data” penalties will **NOT** affect future analyses and do not require retesting
- A 1 is added to the technical score if the data were received late once in 4 rounds of testing, a 2 is added to the technical score if the data is received late twice within 4 rounds of testing
- A late penalty will down-grade your panel score (i.e. a C1 = 2 points)

HIV RNA – The Report

- Table 1- the data included in the analysis
 - The assay and lab ID
 - The panel ID
 - The data exported by the lab
 - The nominal or expected values for the panel
 - The recomputed data – the estimates obtained by NERI in recalculating the data from the raw data
 - Flags associated with the data
 - Note: a flag of 1 is not a penalty, it simply indicates the sample was not detected.

HIV RNA – The Report

TABLE 1. DATA USED IN SCORING
STANDARD ROCHE ASSAY

----- LAB=29 -----

SAMPLE **	NOMINAL CONC	EXPORTED RNA CONC	RECOMPUTED ROCHE ESTIMATED CONC	FLAG
RNA011SM.04A	0	* 201	* 202	1
RNA011SM.02A	500	971	971	
RNA011SM.01A	9,500	16,562	16,562	
RNA011SM.03A	66,500	79,353	79,353	
RNA011SM.05A	332,500	299,536	299,536	
RNA011SM.09A	0	* 114	* 114	1
RNA011SM.08A	500	492	492	
RNA011SM.06A	9,500	8,300	8,300	
RNA011SM.10A	66,500	53,905	53,905	
RNA011SM.07A	332,500	159,567	159,567	
RNA012SM.02A	0	* 225	* 225	1
RNA012SM.01A	500	824	824	
RNA012SM.03A	4,375	5,533	5,533	
RNA012SM.04A	70,000	90,347	90,347	
RNA012SM.05A	280,000	281,620	281,620	
RNA012SM.06A	0	* 270	* 270	1
RNA012SM.09A	500	600	600	
RNA012SM.07A	4,375	3,896	3,896	
RNA012SM.10A	70,000	94,175	94,175	
RNA012SM.08A	280,000	261,654	261,654	

* Below Detection Limit

FLAG = 1 - Valid But Below Detection Limit, 3 - Invalid

** SAMPLE ID CONSISTS OF:

- 3-DIGIT PANEL NUMBER
- R FOR RNA PANEL
- 2 CHARACTERS FOR KIT (SM: STANDARD MONITOR, UM: ULTRASENSITIVE MONITOR,
UB: bDNA VERSION 3, SN: NUCLISENS)
- A FOR FIRST RUN, B FOR SECOND RUN
- 2 DIGITS FOR SAMPLE NUMBER

HIV RNA – The Report

- Table 2 – The breakdown of the precision statistics for the analysis
 - Lab ID
 - Within run precision (intra-assay SD)
 - Between run precision (inter-assay SD)
 - Total assay precision (combination of both intra- and inter-assay precision)
 - The target log₁₀ SD is 0.15. A penalty occurs when a lab's SD is statistically greater.

HIV RNA – The Report

TABLE 2. THE INTRA-ASSAY, INTER-ASSAY AND TOTAL STANDARD DEVIATIONS

THE TOTAL STANDARD DEVIATION IS THE SQUARE ROOT OF THE SUM OF THE SQUARED INTRA-ASSAY AND INTER-ASSAY STANDARD DEVIATIONS

LAB	INTRA ASSAY SD	INTER ASSAY SD	TOTAL SD
29	0.116	0.090	0.15

HIV RNA – The Report

- Table 3 – Accuracy statistics

- The average log₁₀ recovery obtained in the lab's data set
- The average log₁₀ recovery obtained in the field by all labs using the same kit
- The standard error for the data obtained in the field by all labs using the same kit
- The accuracy statistic:
 $(R-M)/S$ where R is average log₁₀ recovery in a laboratory, M is median log₁₀ recovery across all laboratories and S is the standard error of the mean.

HIV RNA – The Report

TABLE 3. AVERAGE LOG10 RECOVERY, THE EXPECTED VALUE OF LOG10 RECOVERY, THE STANDARD ERROR OF LOG10 RECOVERY AND THE ACCURACY STATISTIC

AVG LOG10 RECOVERY IN YOUR LAB	EXPECTED VALUE OF LOG10 RECOVERY	STANDARD ERROR	ACCURACY STATISTIC
0.00570	0.00125	0.06058	0.07349

HIV RNA – The Report

- Table 4 – The summary of the analysis
 - Provides the total assay SD
 - Provides the incidence of false negative (FN) and false positive (FP) results, as well as the number (N) of samples included in the analysis (note: only samples intended for sensitivity are included in this analysis – FN results on ‘other’ specimens would result in a precision problem but would not be documented in FN category)
 - A p-value indicates a penalty; 0.05 indicates a PC, 0.01 indicates a P
 - Recommended score sums up the whole analysis
 - Scores are final AFTER review by QA Subcommittee (QASC)

HIV RNA – The Report

TABLE 4. SCORING SUMMARY USING ACCURACY AND THE TOTAL ASSAY STANDARD DEVIATION. THIS TABLE INCLUDES THE OFFICIAL

LAB	NS	NSD	SD	PSD*	FP	NI	NIC	FN	NDL	FNDL	P_FNDL*	ACCURACY	P_ACC*	RECOMMENDED SCORE
29	20	12	0.15		0	0	0	0	4	0		0.073487		C

[1] NS = number of samples in the series.

[2] NSD = number of positive samples used to calculate SD.

[3] SD = total assay standard deviation of log recoveries

[4] FP = number of neg samples above detection limit

[5] NI = number of invalid results in prepared specimens

[6] NIC = number of incorrectly calculated results in prepared specimens

[7] FN = number of pos samples below detection limit

[8] NDL = number of samples at the limit of detection

[9] FNDL = number of negative results at the limit of detection

[10] ACCURACY = positive values represent the number of standard errors above the median, negative numbers represent the number of standard errors below the median (median defined across all labs using same kit)

[11] PSD, P_FNDL, P_ACC = p-values for SD, FNDL, ACCURACY (blank if p-value > 0.05).

***A p-value (i.e. 0.05 or 0.01) will be displayed only if the result is significantly different than expected and a penalty is assessed (i.e. PC or P)**

HIV RNA – Change in Status

- If a lab changes in approval status this triggers a ‘Change in Status Letter’
 - Cumulative Ratings are reviewed by QASC in a blinded manner before letters are drafted
 - Letter is drafted by the VQA
 - Letter is reviewed QASC chair
 - Letter is sent to lab PI, Network monitors (those individuals selected by the network to monitor QA performance) and DAIDS
 - The VQA **recommends** scores and ratings; Networks impose testing restrictions