Purpose:
The purpose of this document is to provide guidance on the use of VQA HIV RNA copy control sets to evaluate a change in kit lots or kit components that do and do not require recalibration per the manufacturer's specifications.

Background:
Depending on the assay used for HIV RNA testing, there are differences in the way a laboratory should evaluate the performance across kit lots. For the Abbott RealTime HIV-1 RNA assay (AR), it is recommended that the user recalibrate the system whenever the following events occur: 1) a change occurs in the specimen extraction or amplification kit lot, 2) a major repair is performed, 3) a time span of six months has elapsed, or 4) if a problem is suspected. For the COBAS AmpliPrep/COBAS TaqMan HIV RNA assay (RTv2), it is recommended that the user verify the assay performance if any of the following events occurs: 1) there is a change in kit lot, 2) a major repair is performed, or 3) anytime problems are suspected. The use of an external quality control material (QCM) such as the VQA HIV RNA copy control is a useful material to use for evaluating any HIV RNA assay platform because it has a "known" nominal value and it consists of intact viral particles just like a clinical research sample. It is important to test more than one level of control in order to best evaluate assay performance across the reportable range of the assay; as such, the VQA proposes control set that includes the following nominal values: 0, 200, 1,500, 15,000, and 150,000 cp/mL.

The HIV RNA copy controls produced by the VQA consist of a well-characterized virus stock that is diluted in HIV-negative true human serum that contains EDTA (6mM final concentration) and mirrors EDTA plasma in performance on the current HIV RNA assay platforms. Each production is validated against the nominal value (which is defined by serial dilution of the characterized stock) using both the AR and RTv2 HIV RNA assays. The median value of all the testing replicates must fall within +/-1 standard deviation (1 log10 SD = 1.667 log10 RNA cp/mL); this ensures reproducibility of control performance across VQA production lots. For evaluation of assay performance, the testing result should not exceed +/- 0.3 log10 cp/mL; results beyond this value could indicate a significant change in performance that is only attributable to a change in kit lot or a change in assay calibration which is only one component of variability.

Assay performance is not only affected by changes in reagent, but can also be affected by instrument performance. Both optical and mechanical factors can affect instrument performance and assay results. The manufacturer does offer timelines for routine and preventative maintenance schedules, but the user should be aware that problems may begin before those schedule are met, especially in instruments that are getting older (e.g. 10 years old) or in instruments that are used in high throughput settings. These guidelines offer suggestions for the improvement of monitoring HIV RNA assay performance over time; these guidelines are not intended to replace the recommendations of the manufacturer, but instead are tools to help make the user better able to evaluate changes in assay performance.

Calibration Monitoring or Kit Lot Change:

Procedure:
One set of VQA HIV RNA calibration controls (0, 200, 1,500, 15,000, and 150,000 cp/mL) should be included in every run that includes calibrators (AR) or for evaluating a new kit lot performance (RTv2). The nominal value of the control will be used to evaluate assay performance; a +/- 0.3 log10 range will be used to determine acceptability of the result. The acceptable ranges for the VQA calibration controls are provided in Table 1.

The laboratory should include a full set of controls in every calibration run (for Abbott) or in any run that is being performed to evaluate a new kit lot. The results for each control level should be entered into the shaded fields of the spreadsheet (Figure 1, EXCEL file will be provided by the VQA) to determine if

the results are acceptable. Guidelines for interpreting the results from the testing are provided below. A copy of the run file and the spreadsheet should be maintained as part of the laboratory record.

Data Interpretation Guidelines:

Note: These interpretations are intended to be guidelines; this testing is recommended, not required. Feel free to contact the VQA (vqa@rush.edu) for questions regarding these guidelines.

1. If all five of the control levels fall within the acceptable range, then the run is acceptable.
   Note: If there is a trend, e.g., all 4 control levels with a quantitative nominal value are >0.25 log10 RNA cp/mL high or low, the laboratory should consider retesting to see if the trend continues.
2. If one of the control levels exceeds the acceptability criteria, but other levels do not show a trend like that described above, the laboratory may accept the run but should monitor performance of that control level in a subsequent run, especially if the VQA200 control is the affected control, as this can affect run validity in future assays.
3. If two or more of the controls fail the acceptability criteria then the laboratory should troubleshoot the problem (e.g. verify maintenance schedules, instrument performance, technical performance) and retest, recalibrate, or fail a kit lot based on the outcome of the investigation.
4. If a false positive is noted in the VQA0 control, then decontamination procedures should be employed, and contamination checks should be done prior to retesting of several known negative samples to confirm the contamination is removed.

Table 1:

VQA Control Name	VQA Control Nominal Value	Log10 Nominal	Minimum Acceptable Value	Log10 Minimum	Maximum Acceptable Value	Log10 Maximum	Range (Integer)	Range (Log10)
VQA0	0	NA	NA	NA	NA	NA	TND	TND
VQA200	200	2.30	100	2.00	398	2.60	100-398	2.00-2.60
VQA1,500	1,500	3.18	759	2.88	3,020	3.48	759-3,020	2.88-3.48
VQA15,000	15,000	4.18	7,586	3.88	30,200	4.48	7,586-30,200	3.88-4.48
VQA150,000	150,000	5.18	75,858	4.88	301,996	5.48	75,858-301,996	4.88-5.48

Figure 1: VQA HIV RNA Control Testing Worksheet

	SOP#								v.20161129			
	Appendix A:		QUANTITATIVE HIV RNA CONTROL TESTING WORKSHEET									
			enter your laboratory name here									
			Quantitative PCR Reagent lot-to-lot (Parallel) Testing Worksheet									
	PCR Test/Kit:					Manufacturer:						
	Old Kit:											
	Lot number:			**Expiration Date:**								
	New Kit:											
	Lot Number:			**Expiration Date:**								
	Name of Technologist performing testing:							**Date:**				
	PURPOSE OF TESTING (e.g. kit lot comparison or calibration verification):											

CONTROL INFORMATION			**NOMINAL VALUE**			**New kit**			**Acceptability criteria**			
No.	**Sample ID**	**CONTROL LOT #**	**NOMINAL VALUE**	**VL Results (Log10)**	**Acceptable Range (+/-)**	**Assay Date**	**VL Results (Integer)**	**VL Results (Log10)**	**Lower limit** (LOG NOMINAL - 0.3)	**Upper limit** (LOG10 NOMINAL + 0.3)	**-0.3 LOG ACCEPTABILITY**	**+0.3 LOG ACCEPTABILITY**
1	VQA0		1	0.0000	0.3000		100	2.0000	-0.3000	0.3000	**NOT**	**NOT**
	VQA200		200	2.3010	0.3000		500	2.6990	2.0010	2.6010	**ACCEPTABLE**	**NOT**
2	VQA1,500		1,500	3.1761	0.3000		750	2.8751	2.8761	3.4761	**NOT**	**ACCEPTABLE**
3	VQA15,000		15,000	4.1761	0.3000		7,500	3.8751	3.8761	4.4761	**NOT**	**ACCEPTABLE**
4	VQA150,000		150,000	5.1761	0.3000		75,000	4.8751	4.8761	5.4761	**NOT**	**ACCEPTABLE**

**key in the result of "Target Not Detected" as "1"*

Please attach the printed raw data as part of the testing record

Supervisor Review; Name:…….. Signature:…….. Date:……..

QA/QC Review; Name:…….. Signature:…….. Date:……..

DLS Approval; Name:…….. Signature:…….. Date:……..

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