



New HIV-1 RNA Assay Validation: **Roche COBAS TaqMan and Abbott RealTime**

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VQA CROSS PLATFORM ANALYSIS

- **Primary Purpose:** To determine the comparability and overall performance of new RNA sample processing systems (Abbott m2000sp & Ampliprep) and RNA assays (Abbott m2000rt, Roche COBAS TaqMan 48) to existing assays.
- **Secondary Purpose:** To provide laboratories a validation method which can be used during the installation of a new RNA Assay.

Abbott M2000 System

the *New M2000*
RealTime PCR System



m2000sp

m2000rt



Roche COBAS TaqMan



COBAS AmpliPrep



COBAS TaqMan 48



COBAS AmpliPrep/TaqMan 96



Primary Validation

- Phase 1:
 - HIV-1 Spiked Panels
- Phase 2:
 - HIV-1 Clade Spiked Panels
- Phase 3:
 - Patient Specimens



Phase One

RNA001XP (45)	
Cps/mL	Replicates
0	5
10	4
20	4
40	5
80	5
175	5
350	4
1,000	3
5,000	3
50,000	3
250,000	2
500,000	2

RNA002XP (21)	
Cps/mL	Replicates
0	2
50,000	2
250,000	3
500,000	4
1,000,000	5
2,000,000	5

RNA005/6XP (21)	
Cps/mL	Replicates
0	1
2.5	2
5	2
10	2
20	2
40	3
50	3
100	2
200	2
400	2

Proportion With Quantitative Results

Nom. Conc. (cp/mL)	AR	RT1	RU-COB
25	16.7% (4/24)	58.3% (7/12)	NA (0/4)
40	30.3% (44/145)	73.1% (106/145)	(6/35)
50	52.2% (109/209)	90.6% (202/223)	17.1% (110/456)
80	60% (15/25)	100% (25/25)	80% (4/5)
100	93.8% (75/80)	100% (80/80)	90% (18/20)
175	100% (25/25)	100% (25/25)	100% (5/5)
200	100% (80/80)	100% (80/80)	100% (20/20)
250	100% (25/25)	100% (36/36)	100% (24/24)
350	100% (20/20)	100% (20/20)	100% (4/4)
400	100% (80/80)	100% (80/80)	100% (20/20)
500	100% (8/8)	100% (17/17)	100% (120/120)

Results detected but less than the limit of detection are not included in the numerator. The ratio in brackets represents the number of samples with a quantitative result over the total number of samples at that concentration. Quantitative detection rates in bold represent the highest concentration with less than 95% quantitatively detected results.



Total-Assay Standard Deviations

Nom. Conc. (cp/mL)	AR	RT1	RU-COB
25	0.07	0.23	NA
40	0.12	0.16	0.12
50	0.14	0.18	0.24
80	0.14	0.16	0.13
100	0.17	0.13	0.17
175	0.14	0.13	0.04
200	0.15	0.13	0.17
250	0.18	0.15	0.19
350	0.07	0.10	0.11
400	0.12	0.10	0.15
500	0.13	0.12	0.20

Total assay standard deviations in bold represent the highest concentration with less than 95% quantitatively detected results.

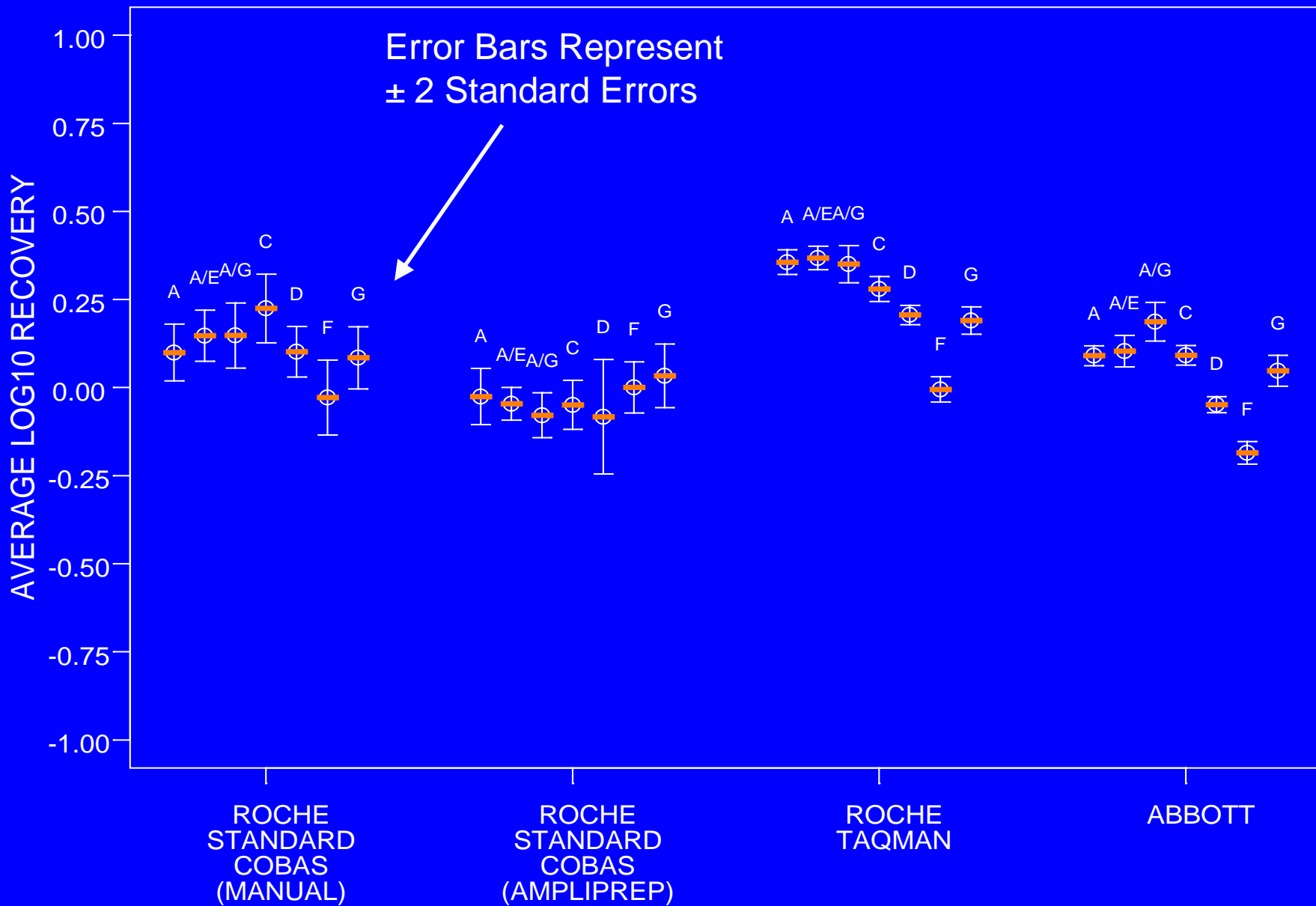


Phase Two

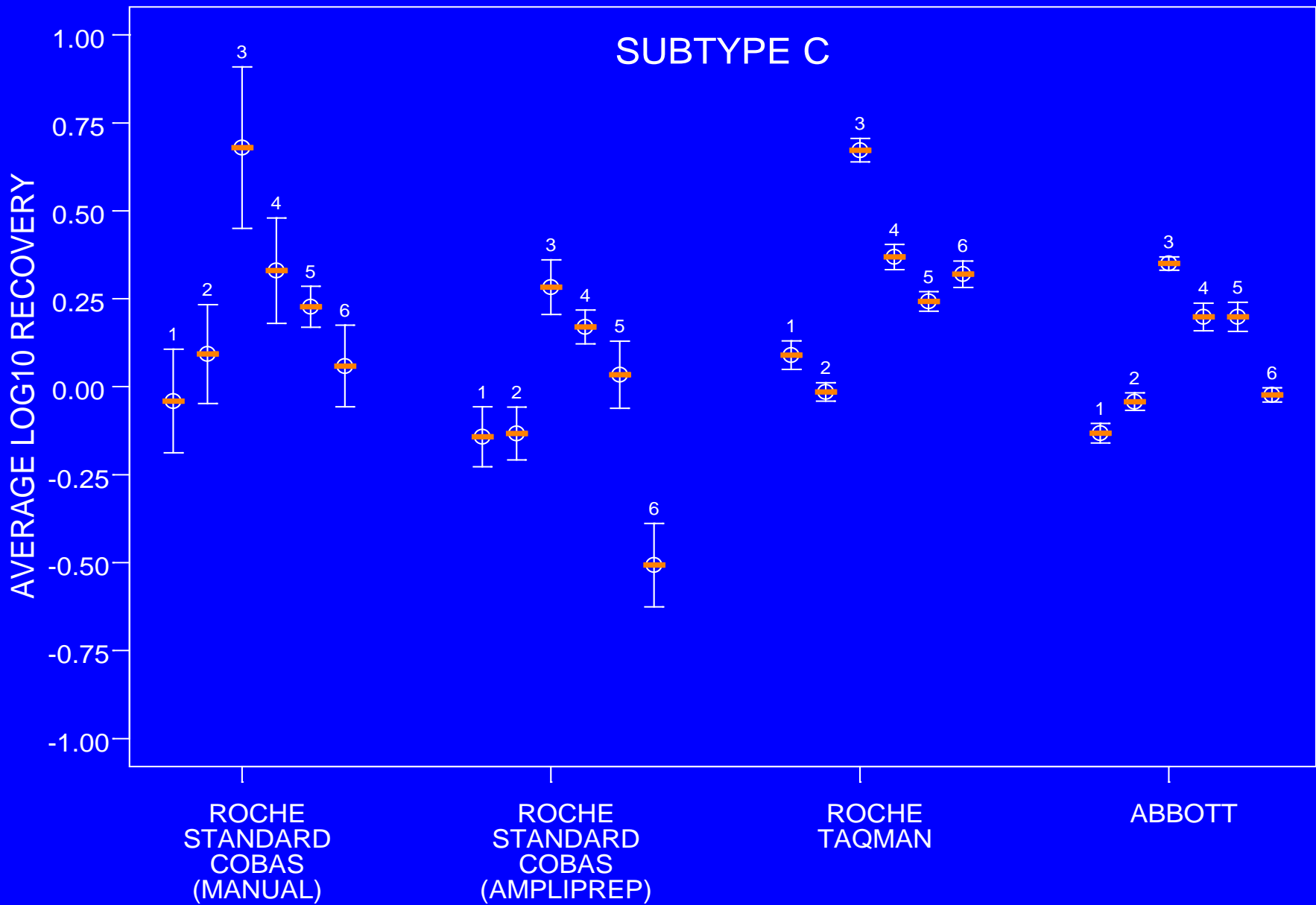
- **Each Phase Two Panel Will Consist of:**
 - 28 HIV non-B Clade Samples
2-A, 7-A/E, 3-A/G, 6-C, 3-D, 5-F, 2-G
 - Each Tested at Two Concentrations
2,500 cps/mL and 25,000 cps/mL
 - Each Concentration Tested in Triplicate
 - 168 Total Samples per Panel
- **Each Phase Two Panel will be tested 1x or 2x on The Original Assays**
- **Each Phase Two Panel will be tested 5x on The New Real-Time Assays**

Clade	ID	Clade	ID
A	UG273	C	SM145
A	UG275	C	SE364
A/E	CM235	C	UG268
A/E	NP1565	C	DJ259
A/E	NP03	C	ETH2220
A/E	POC30506	C	ZAM18
A/E	ID17	D	SE365
A/E	42368	D	UG270
A/E	ID12	D	UG274
A/G	DJ258	F	BCI-R07
A/G	POC44951	F	BCI-R08
A/G	DJ263	F	BZ126
G	BCF-DIOUM	F	BZ162
G	HH8793	F	BZ163

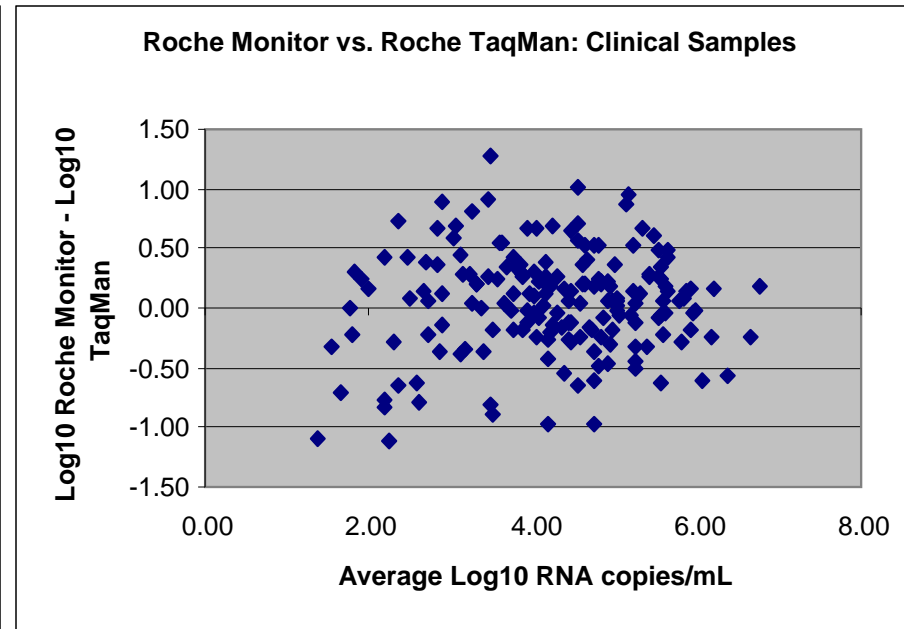
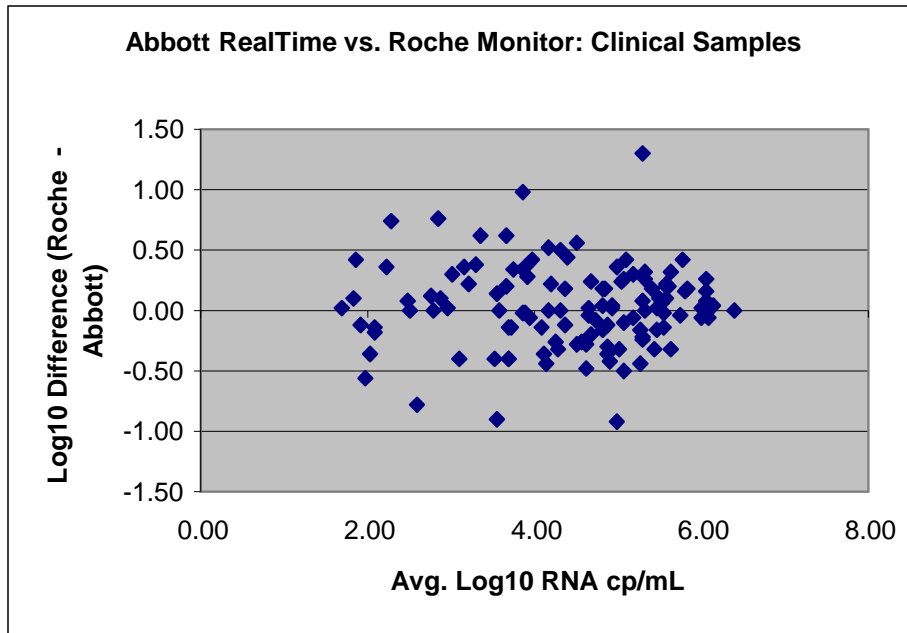
AVERAGE LOG10 RECOVERY BY PLATFORM



AVERAGE LOG10 RECOVERY BY STRAIN AND PLATFORM



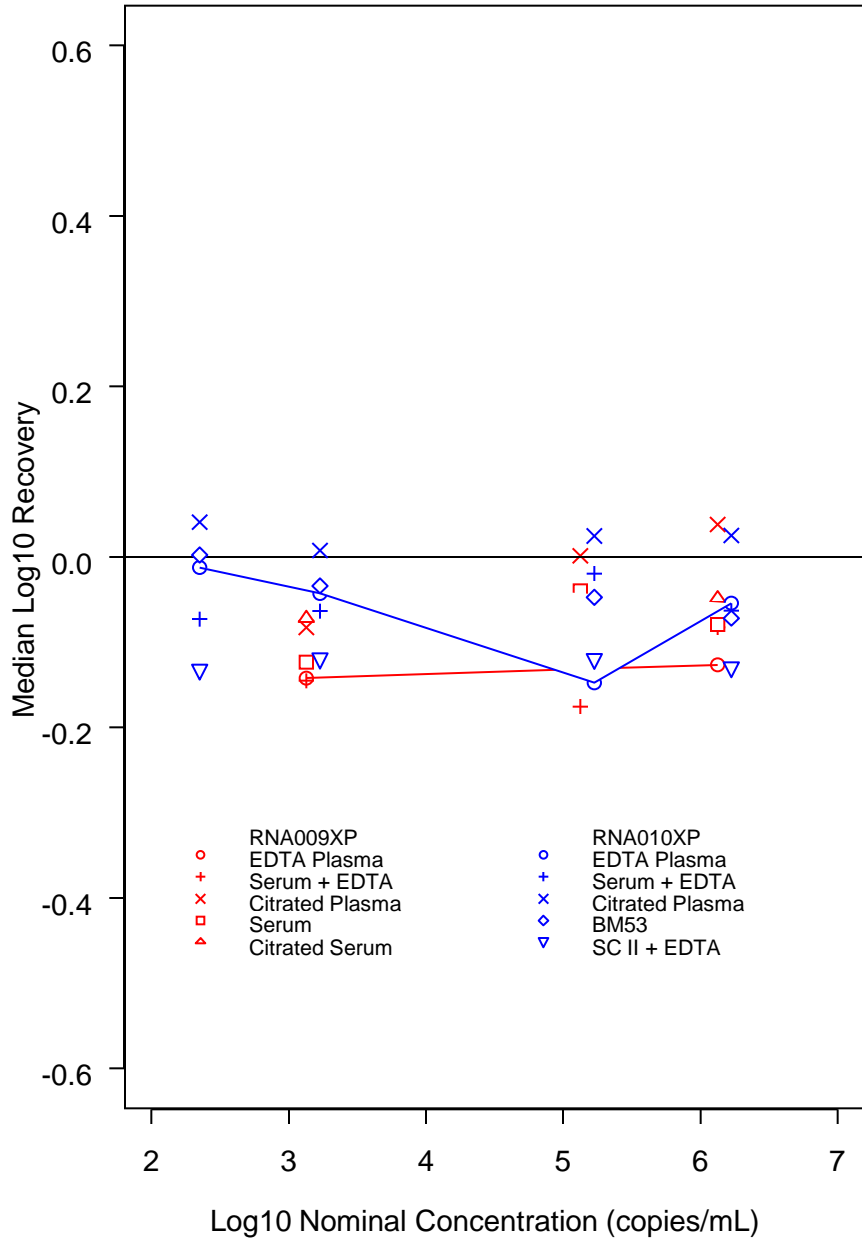
Patient Specimens



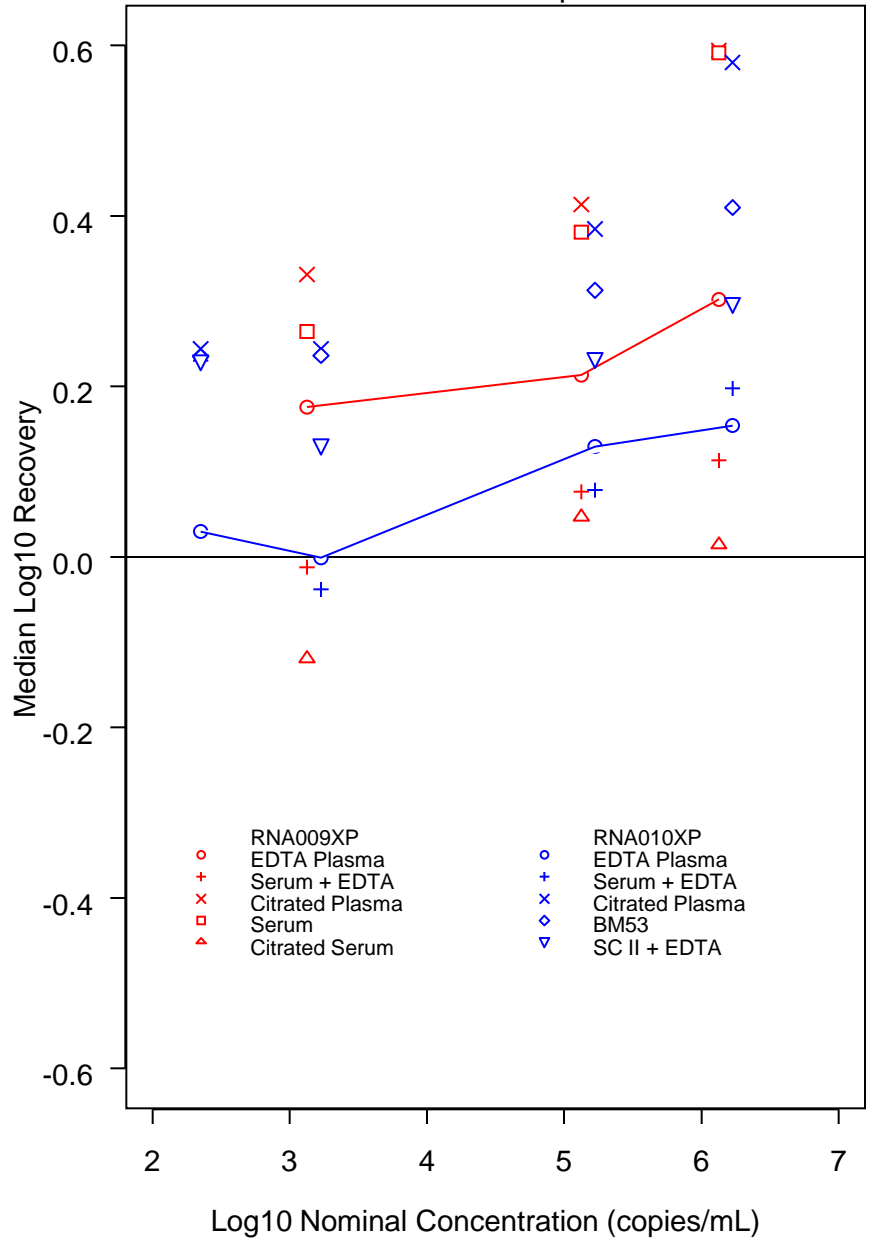
Median Log10 Recovery vs. Log10 Nominal Concentration

Data From VQA Laboratory 11/2009, 4 Replicates Per Median

Abbott Real Time



Roche TaqMan v1





VQA HIV RNA ASSAY VALIDATION:

Purpose

- To evaluate the installation of a new HIV RNA assay into the laboratory
- To compare the results obtained on the “new” assay to the results obtained on the “current” HIV RNA platform



VQA HIV RNA ASSAY VALIDATION: Materials

- VQA Validation Template and SOP
- VQA RNA Copy Controls
- 20 HIV- donors
- 25-39 HIV+ donors
 - Sufficient volumes to run on two assay and permit repeats if necessary
 - 80% should have detectable virus loads



VQA HIV RNA ASSAY VALIDATION: Data Collection

- 5 runs of 24 samples (incl. 3 kit controls) per HIV RNA assay
- Data collected over five days on each platform
- Results transcribed to excel file
 - All samples must be valid
 - Samples above the upper LOD must be diluted and repeated
 - Invalid results must be repeated
 - Raw data files should be saved for review

VQA HIV RNA ASSAY VALIDATION

Validation Assay Templates

Run #1	Run #2*	Run #3	Run #4	Run #5
NC	NC	NC	NC	NC
LPC	LPC	LPC	LPC	LPC
HPC	HPC	HPC	HPC	HPC
50	50	50	50	50
1500	1500	1500	1500	1500
15000	15000	15000	15000	15000
150000	150000	150000	150000	150000
50	1500000	1500000	1500000	1500000
1500	SN Donor 1	SN Donor 2	SN Donor 3	SN Donor 4
15000	Donor 1	Donor 9	Donor 18	Donor 29
150000	Donor 2	Donor 10	Donor 19	Donor 30
50	Donor 3	Donor 11	Donor 20	Donor 31
1500	1500000	Donor 12	Donor 21	Donor 32
15000	SN Donor 5	SN Donor 6	SN Donor 7	SN Donor 8
150000	Donor 4	25	Donor 22	Donor 33
50	Donor 5	Donor 13	Donor 23	Donor 34
1500	1500000	Donor 14	Donor 24	Donor 35
15000	SN Donor 9	SN Donor 10	SN Donor 11	SN Donor 12
150000	Donor 6	Donor 15	Donor 25	Donor 36
25	Donor 7	Donor 16	Donor 26	Donor 37
1500	SN Donor 13	SN Donor 14	SN Donor 15	SN Donor 16
15000	Donor 8	Donor 17	Donor 27	Donor 38
150000	1500000	25	Donor 28	Donor 39
25	SN Donor 17	SN Donor 18	SN Donor 19	SN Donor 20

Run #1	Run #2	Run #3	Run #4	Run #5
NC	NC	NC	NC	NC
LPC	LPC	LPC	LPC	LPC
HPC	HPC	HPC	HPC	HPC
50	50	50	50	50
1500	1500	1500	1500	1500
15000	15000	15000	15000	15000
150000	150000	150000	150000	150000
50	1500000	1500000	1500000	1500000
1500	SN Donor 1	SN Donor 2	SN Donor 3	SN Donor 4
15000	Donor 1	Donor 9	Donor 18	Donor 29
150000	Donor 2	Donor 10	Donor 19	Donor 30
50	Donor 3	Donor 11	Donor 20	Donor 31
1500	1500000	Donor 12	Donor 21	Donor 32
15000	SN Donor 5	SN Donor 6	SN Donor 7	SN Donor 8
150000	Donor 4	25	Donor 22	Donor 33
50	Donor 5	Donor 13	Donor 23	Donor 34
1500	1500000	Donor 14	Donor 24	Donor 35
15000	SN Donor 9	SN Donor 10	SN Donor 11	SN Donor 12
150000	Donor 6	Donor 15	Donor 25	Donor 36
25	Donor 7	Donor 16	Donor 26	Donor 37
1500	SN Donor 13	SN Donor 14	SN Donor 15	SN Donor 16
15000	Donor 8	Donor 17	Donor 27	Donor 38
150000	1500000	25	Donor 28	Donor 39
25	SN Donor 17	SN Donor 18	SN Donor 19	SN Donor 20



VQA HIV RNA ASSAY VALIDATION: Analyses

- Precision – VQA RNA Controls
 - Linear analysis - %CV (target 35%)
 - Log10 transformed data – SD (target 0.15)
- Accuracy – VQA RNA Controls
 - Bland-Altman plots (log10 difference vs log10 nominal)
 - Pearson's Correlation (target >0.95)
- Reportable Range – VQA RNA Controls
 - Bland-Altman plots (log10 difference vs log10 nominal)
- Carryover – High+ VQA Controls + HIV-
- Correlation – HIV+ Donor Samples
 - Log10 RNA (Assay 1) vs Log10 RNA (Assay 2)
 - Bland-Altman plots (log10 difference vs log10 average)



Acknowledgments

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