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PERFORMANCE EVALUATION OF THE APTIMA HIV-1 QUANT ASSAY ON THE PANTHER SYSTEM

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BACKGROUND

- In the U.S., the APTIMA HIV-1 Quant Assay (APT-Quant) is FDA-approved only for quantification of HIV-1 plasma RNA from 1.47-7 log(copies/ml)
- Outside of the U.S., the assay is also approved for HIV diagnosis



OBJECTIVE

To evaluate the performance of the APT-Quant for HIV-1 RNA quantification in different sample sets and for diagnosis use (off-label in the US)

HIV SAMPLES and METHODS

- Plasma specimens were tested with APT-Quant, and volume permitting, with APTIMA HIV-1 RNA Qualitative (APT-Qual), Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test v2.0 (Roche viral load (VL)), and Abbott RealTime HIV-1 (Abbott VL) assays
- AccuSpan HIV-1 RNA linearity panels (LP) were used to measure agreement between VL assays and reproducibility over 3 days with 2 kit lots
- 420 samples from 46 US seroconverters (subtype B) and 113 Cameroonian HIV-1 antibody-positive samples (110 non-B subtype - gp41: A, CRF02_A, CRF01_E, D, F2, G- and 3 Group O) were used to measure agreement and APT-Quant as a diagnostic assay
- 478 HIV-negative samples (APT-Qual negative/HIV-1 antibody-negative) were used to measure specificity
- Agreement by linear regression and concordance correlation coefficient; test reproducibility by standard deviation and McNemar's paired test to compare with APT-Qual

RESULTS

Table 1 - Reproducibility of APT-Quant with HIV-1 RNA linearity panels

	Roche VL log (cop/ml)	Abbott VL log (cop/ml)	Panther APT-Quant (lot#1) log (cop/ml) day 1	log (cop/ml) day 2	log (cop/ml) day 3	mean	standard deviation	Panther APT-Quant (lot#2) log (cop/ml) day 1	log (cop/ml) day 2	log (cop/ml) day 3	mean	standard deviation
LP 01	7.91	>7	>7.00	>7.00	>7.00	>7		invalid*	invalid*	invalid*		
LP 02	6.72	6.75	>7.00	>7.00	>7.00	>7		>7.00	>7.00	>7.00	>7	
LP 03	5.85	5.72	6.31	6.32	6.28	6.3	0.02	6.35	6.34	6.32	6.33	0.02
LP 04	4.96	4.69	5.19	5.19	5.04	5.14	0.09	5.24	5.19	5.25	5.22	0.03
LP 05	4.22	3.76	4.00	4.07	4.03	4.03	0.04	4.1	4.13	4.11	4.11	0.02
LP 06	3.07	2.71	2.91	2.88	2.7	2.83	0.11	2.84	2.64	3.57	3.02	0.49
LP 07	1.91	1.97	<1.47	1.75	1.61			1.97	1.85	1.71	1.84	0.13
LP 08	<1.3	TND	<1.47	<1.47	TND			TND	TND	<1.47		
LP 09	TND	TND	TND	TND	TND			TND	TND	TND		
LP NEG	TND	TND	TND	TND	TND			TND	TND	TND		

- Invalid results were observed when high HIV-1 RNA concentrations inhibit amplification of the Internal Control
  - In this case, samples should be diluted 1:100 as per package insert for quantification
- The highest inter-run variability was observed with LP 06 with concentrations ~ 3 log (cop/ml)
  - Samples were tested in singlet
- APT-Quant quantified most of LP07 with concentrations ~ 1.94 log (cop/ml) or 87 cop/ml (highlighted in red)

Table 2 - Comparison between APT-Quant and FDA-approved VL Assays in different sample sets

HIV-1 US Seroconverters

Panther APT-Quant	Roche HIV-1 v2.0 TND	< 1.3 cop/ml	x cop/ml
TND	43	2	3
< 1.47 cop/ml	18	2	7
x cop/ml	4	0	150

Panther APT-Quant	Abbott m2000 HIV-1 TND	< 1.6 cop/ml	x cop/ml
TND	42	4	0
< 1.47 cop/ml	27	18	3
x cop/ml	8	14	255

HIV-1 Cameroonian Established Infections

Panther APT-Quant	Roche HIV-1 v2.0 TND	< 1.3 cop/ml	x cop/ml
TND	0	0	0
< 1.47 cop/ml	0	0	3
x cop/ml	1	2	95

Panther APT-Quant	Abbott m2000 HIV-1 TND	<1.6 cop/ml	x cop/ml
TND	0	0	1
< 1.47 cop/ml	0	0	3
x cop/ml	0	1	98

	Linearity Panel Kit Lot #1*	Linearity Panel Kit Lot #2*	HIV-1 US Early Infections	HIV-1 Cameroonian Established Infections
APT-Quant vs Roche				
Sample size of quantifiable values	4	5	150	95
Linear regression (R^2)	0.994	0.993	0.936	0.766
Concordance correlation coefficient	0.9696	0.9857	0.9397	0.8348
95% Confidence interval	0.9233 - 0.9881	0.9423 - 0.9965	0.9203 -0.9545	0.7703 - 0.8823
Pearson ρ (precision)	0.9972	0.9965	0.9674	0.8751
APT-Quant vs Abbott				
Sample size of quantifiable values	4	5	255	98
Linear regression (R^2)	0.999	0.996	0.881	0.869
Concordance correlation coefficient	0.9483	0.9597	0.9245	0.9204
95% Confidence interval	0.7052 - 0.9919	0.8249 - 0.9912	0.9048 -0.9402	0.8851 - 0.9452
Pearson ρ (precision)	0.9999	0.9978	0.9387	0.9324

- *R^2 (Roche vs Abbott)= 0.987 for HIV-1 RNA LPs and 0.7376 for HIV-1 Cameroonian samples (n=91)
- For US and Cameroonian samples the agreement analysis was done using singlet results and VL > 7 log (cop/ml) was considered as 7

Table 3 - APT-Quant for Diagnostic Use

HIV-1 US Seroconverters		APT-Qual	
		Reactive	Nonreactive
APT-Quant	Reactive	328	34
	Nonreactive	7	48

- For the analysis all APT-Quant detected results were considered HIV-1 RNA Reactive
- In HIV-1 seroconversion panels:
  - ✓ APT-Quant significantly detected virus in more samples than APT-Qual (n=417, *p<0.0001*)
  - ✓ In 228 seroconversion samples collected very early after infection, APT-Quant detected virus in 180 (80%), Roche VL in 163 (71.5%), and APT-Qual in 157 (68.9%)
  - ✓ APT-Qual and APT-Quant missed two infections with Roche <1.3 log (cop/ml) and one with 1.37 log (cop/ml)
- All Cameroonian established infections were detected with APT-Quant and APT-Qual assays

SUMMARY

- APT-Quant shows reliable performance in different sample sets with low inter-run variability in most cases
- APT-Quant specificity was 99.79% [CI: 98.82- 99.96%]
- Although the limit of detection was not estimated, in HIV-1 seroconverters where assay analytical sensitivity is challenged, APT-Quant performed very well for detection and quantification of HIV-1 RNA
- Agreement between APT-Quant and other FDA-approved VL assays was better in US samples compared to samples from Cameroon where non-B subtypes are present
- APT-Quant was more sensitive than other HIV-1 RNA tests at detecting virus in samples collected early after HIV-1 infection

CONCLUSIONS

- APT-Quant performs with high sensitivity and specificity
- APT-Quant performance exceeds APT-Qual, the only FDA-approved diagnostic assay, for detecting HIV-1 infections with low HIV-1 RNA levels
- The APT-Quant assay could also be used for diagnostics if properly validated in US laboratories or via an FDA diagnostic claim

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