Performance Evaluation of the Aptima® HIV Quant Dx and Aptima® HBV Quant assays on the fully automated Panther in comparison to COBAS® AmpliPrep/ COBAS® TaqMan® HIV-1 and HBV tests

biomnis SPECIALISED MEDICAL PATHOLOGY

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Background

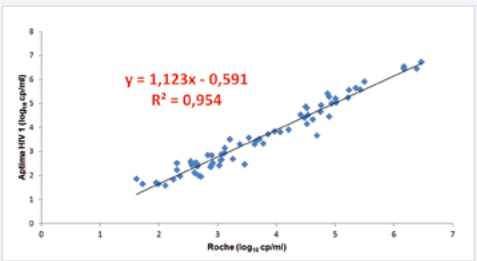
Quantification of HIV-1 RNA and HBV DNA viral load plays a central role in clinical management of HIV and HBV infected patients, before and during antiviral therapy.
The Hologic Aptima® HIV-1 Quant Dx assay and HBV Quant assay are commercially available quantitative assays, using 0.5ml sample input for use on the Panther system. The assays are based on real-time Transcription Mediated Amplification (TMA) technology.

Assay performance

Method comparison

HIV 191 plasma samples (94 prospective and 97 retrospective) from HIV-1 infected patients were tested using Aptima® HIV-1 Quant Dx Assay, based on HIV viral load, as determined by routine testing using COBAS® TaqMan® HIV-1 test.

Fig. 1 Comparison of Hologic and Roche assays (n=67)



Deming regression was excellent between the 2 assays for quantifiable samples: y = 1.12 x -0.59, R^2 = 0.954

Tab 1. Agreement between Roche and Hologic assays for detection and quantification of HIV RNA

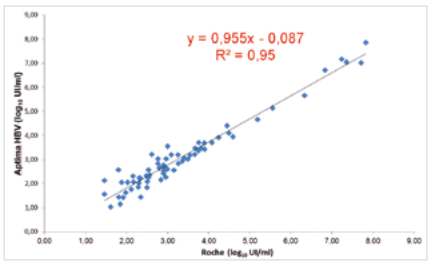
		HIV1 Quant Dx Aptima cp/ml			
		TND	Detected < 30	Quantified	Total
Roche HIV1 cp/ml	TND	58	13	0	**71**
	Detected < 20	13	15	0	**28**
	Quantified	5	20	67	**92**
	Total	**76**	**48**	**67**	**191**

TND: target not detected

The overall percentage of agreement was 73.3%.
Kappa: 0.6
Discrepant results were observed at very low viral load as previously reported [1]

HBV 200 plasma or sera samples (100 prospective and 100 retrospective) from HBV-infected patients were tested for Aptima® HBV Quant Assay, based on HBV viral load, as determined by routine testing using COBAS® TaqMan® HBV test.

Fig. 2 Comparison of Hologic and Roche assays (n=132)



Deming regression was excellent between the 2 assays for quantifiable samples: y = 0.95 x -0.09, R^2 = 0.95

Tab 2. Agreement between Roche and Hologic assays for detection and quantification of HBV DNA

		HBV Quant Aptima UI/ml			
		TND	Detected < 10	Quantified	Total
Roche HBV UI/ml	TND	30	2	0	**32**
	Detected < 20	8	15	1	**24**
	Quantified	0	8	132	**140**
	Total	**38**	**25**	**133**	**196**

TND: target not detected

We observed a very good correlation between the 2 assays
The overall percentage of agreement was 90.3%.
Kappa: 0.8

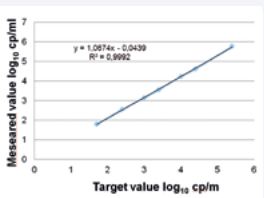
Analytic performance with reference panel

HIV **Qnostics HIV-1 50904 and Bio QControl P0043 HIV-RNA** were used to assess reproductibility and precision

Tab 3. Qnostics HIV1 panel results

Genotype	Target concentration (log_{10} cp/ml)	N	Observed Mean log_{10}	Observed SD log_{10}	Difference (observed-target)
HIV 1 Type B	4,4	3	4,51	0,05	0,11
HIV 1 Type B	3,4	3	3,34	0,08	-0,06
	3,4	2	3,40	0,05	0,00
HIV 1 Type B	2,4	2	2,45	0,02	0,05
HIV 1 Type C	4,4	2	4,56	0,09	0,16
HIV 1 Type C	3,4	2	3,49	0,06	0,09
HIV 1 Type C	2,4	2	2,27	0,12	-0,13

Fig 3. Linearity with Bio QControl P0043HIV-RNA



- Mean difference between measured and expected values was < 0.16 log cp/ml for the Qnostic positive control and < 0.37 log cp/ml for serial dilution of Bio QControl P0043 HIV-RNA (5.40 to 1.70 log) tested in triplicate.
- Standard Deviation (SD) was less than 0.19 for an expected titer of 50 UI/ml for Bio Qcontrol P0043, tested in triplicate.

HIV **Tab 6. Linearity with dilution of S1003 HIV-DOM 04-20047 panel**

Nominal titer (cp/mL)	Nominal concentration (log_{10} cp/ml)	N tested	N detected	N quantified	Observed mean (log_{10} cp/ml)	Observed SD (log_{10} cp/ml)	Difference (observed-nominal)
1000	3	10	10	10	3,19	0,11	0,19
100	2	10	10	10	2,26	0,12	0,26
50	1,70	10	10	10	1,98	0,22	0,28
25	1,40	10	10	7	1,7	0,16	0,3
12,5	1,10	10	10	0	-	-	-
6,25	0,80	10	6	1	1,59	-	0,79
3,125	0,49	10	4	0	-	-	-

Sensitivity assessed with serial dilution of S1003 HIV-RNA DOM 04-20047 panel was 12.5 cp/ml for 100% of 10 replicates. 3.125 cp/ml was found to be positive in 4 out of 10.

HBV **Qnostics 14038 HBV and Bio QControl P0041 HBV DNA** were used to assess reproductibility and precision

Tab 4. Qnostics 14038 HBV panel results

		Expected values log_{10} UI/ml	Measured values log_{10} UI/ml			
Panel specimen	Geno-type	Target value	R1	R2	Mean	Difference (measured-expected)
1438D17	D	1,70	1,32	1,35	1,34	-0,36
1438D37	D	3,70	3,41	3,46	3,44	-0,26
1438A27	A	2,70	2,83	2,70	2,77	0,07
1438A37	A	3,70	3,69	3,64	3,67	-0,03
1438A27	A	2,70	2,73	2,74	2,74	0,04
1438A17	A	1,70	1,77	1,70	1,74	0,04
1438D27	D	2,70	2,55	2,54	2,55	-0,15

Tab 5. Bio QControl P0041 panel results

Expected values log_{10} UI/ml	Measured values log_{10} UI/ml					
	R1	R2	R3	Mean values	Observed SD	Δ
6	5,67	5,85	5,63	5,72	0,12	0,28
5	4,61	4,66	4,55	4,61	0,05	0,39
4	3,77	3,76	3,60	3,71	0,09	0,29
3	2,76	2,63	2,66	2,68	0,07	0,32
2	1,59	1,49	1,41	1,50	0,09	0,5
1	<1,0	<1,0	<1,0	-	-	-

- Genotype A and D were quantified with good accuracy using Qnostics panel (mean difference < 0.36 log_{10}/ml for 50 UI/ml concentration).
- Serial dilution of Bio QControl tested in triplicate were measured with very good accuracy (SD values < 0.12 log_{10}/ml).

HBV **Tab 7. Linearity with dilution of Bio QControl panel**

Nominal titer (UI/ml)	Nominal concentration (log_{10} UI/ml)	N tested	N detected	N quantified	Observed mean (log_{10} UI/ml)	Observed SD (log_{10} UI/ml)	Difference (observed-nominal)
10 000	4	10	10	10	4,06	0,08	0,06
1000	3	10	10	10	3,21	0,06	0,21
100	2	10	10	10	2,23	0,11	0,23
50	1,7	10	10	10	1,87	0,13	0,17
25	1,4	10	10	10	1,56	0,13	0,16
12,5	1,1	10	10	9	1,2	0,12	0,1
6,25	0,8	10	10	1	1,04	-	0,24
3,125	0,49	10	10	0	-	-	-
1,56	0,19	10	10	0	-	-	-

Sensitivity determined with serial dilution of Bio QControl panel was 1.56 IU/ml for 100% of 10 replicates.

Cross contamination

No cross contamination (neither with HIV nor with HBV) was observed when testing 5 consecutive runs using negative control and High positive control samples alternately.

Conclusions

The Hologic Aptima® HIV-1 Quant Dx assay and Aptima® HBV Quant assays as performed on the fully automated Panther system gave highly comparable performance to that of Roche COBAS® TaqMan® HIV-1 v2 and HBV v2.0 assays for clinical samples. Excellent results were observed using commercially available panels indicating high sensitivity and very good reproducibility.

This system, using 0.5 ml sample input on primary samples, was easy to use and could generate 120 test results in less than four hours.

(1) Ref. L.C. Swenson et al. Comparative performance of HIV-1 RNA load assays at low viral load levels: Results of an international collaboration. JCM, Feb 2014, Vol 52, n°2, 517-523

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