Gunnar Schalasta*, Anna Börner, Andrea Speicher and Martin Enders

Evaluation of the Aptima HBV Quant assay vs. the COBAS TaqMan HBV test using the high pure system for the quantitation of HBV DNA in plasma and serum samples

Abstract

Background: Proper management of patients with chronic hepatitis B virus (HBV) infection requires monitoring of plasma or serum HBV DNA levels using a highly sensitive nucleic acid amplification test. Because commercially available assays differ in performance, we compared herein the performance of the Hologic Aptima HBV Quant assay (Aptima) to that of the Roche Cobas TaqMan HBV test for use with the high pure system (HPS/CTM).
Methods: Assay performance was assessed using HBV reference panels as well as plasma and serum samples from chronically HBV-infected patients. Method correlation, analytical sensitivity, precision/reproducibility, linearity, bias and influence of genotype were evaluated. Data analysis was performed using linear regression, Deming correlation analysis and Bland-Altman analysis.
Results: Agreement between the assays for the two reference panels was good, with a difference in assay values vs. target <0.5 log. Qualitative assay results for 159 clinical samples showed good concordance (88.1%; $\kappa = 0.75$; 95% confidence interval: 0.651–0.845). For the 106 samples quantitated by both assays, viral load results were highly correlated ($R = 0.92$) and differed on average by 0.09 log, with 95.3% of the samples being within the 95% limit of agreement of the assays. Linearity for viral loads 1–7 log was excellent for both assays ($R^2 > 0.98$). The two assays had similar bias and precision across the different genotypes tested at low viral loads (25–1000 IU/mL).
Conclusions: Aptima has a performance comparable with that of HPS/CTM, making it suitable for use for HBV infection monitoring. Aptima runs on a fully automated platform (the Panther system) and therefore offers a significantly improved workflow compared with HPS/CTM.

*Corresponding author: Gunnar Schalasta, Prof. Gisela Enders and Kollegen, MVZ GbR Rosenbergstr. 85, 70193 Stuttgart, Germany, Phone: +0711 6357 140, Fax: +0711 6357 148,
E-mail: schalasta@labor-enders.de
Anna Börner, Andrea Speicher and Martin Enders: Prof Gisela Enders and Kollegen, MVZ GbR, Stuttgart, Germany

Introduction

Chronic infection with the hepatitis B virus (HBV) affects an estimated 240 million people worldwide [1]. Oral antiviral treatments such as tenofovir or entecavir have revolutionized the treatment of chronic hepatitis B because they are highly effective in suppressing HBV DNA replication, seldom lead to drug resistance, have simple regimen (one pill a day) and have few side effects. However, they do generally result in a so-called functional cure because they do not eliminate covalent closed circular DNA or viral DNA integrated into the host genome.

Treatment success or failure is assessed by biochemical, serological, virological and histological end points, with the ultimate goal of therapy being a reduction of HBV DNA levels to undetectable levels, ALT normalization, HBsAg loss and HBeAg seroconversion [1–3]. However, because these ideal end points are infrequently achievable altogether with the currently available antiviral agents, a more realistic goal is the induction of sustained virological suppression [2]. To this end, quantitative measurement of HBV DNA levels (viral load) in the plasma/serum has become a key test for confirmation of HBV infection and monitoring of HBV-infected patients during therapy. Guidelines recommend monitoring of HBV DNA levels with highly sensitive nucleic acid amplification technologies every 3 months until HBV DNA is undetectable, and then every 3–6 months thereafter to allow detection of persistent viremia and virological breakthrough [2, 3]. Treatment failure is defined as the inability of an antiviral agent to reduce HBV DNA levels by $\geq 1 \times$ log IU/mL within 3 months of initiating therapy [1]. Virological breakthrough (rebound) is defined as (i) an increase in HBV DNA by ≥1 log compared with the patient's nadir [1–3] or (ii) an increase in HBV DNA level from undetectable levels (<10 IU/mL) to ≥100 IU/mL [3] in HBV-positive patients undergoing therapy.

There are currently many commercially available, real-time nucleic acid amplification tests (NAATs) for HBV DNA quantitation in patients' clinical samples, most of

which using PCR technology. Although these assays are recognized for their high performance, for which they have been granted approval in many countries, studies published in the last decades that have conducted independent assay evaluations and side-by-side comparisons have shown that they slightly vary in sensitivity, bias, precision/reproducibility and degree of automation [4–13].

The Aptima® HBV Quant assay (Aptima; Hologic Inc., San Diego, CA, USA), which was released in late 2015, is the latest NAAT for quantitation of HBV DNA in patients' plasma and serum samples. The Aptima assay runs on the Panther system (Hologic Inc.), a fully automated and integrated platform that completely removes manual processing. The Aptima assay consists of three steps – target capture, target amplification via transcription-mediated amplification (TMA) and real-time detection of amplicons by fluorescent probes – all taking place in a single tube in an automated manner on the Panther platform.

There are limited data currently available on the performance of the Aptima assay compared with other commercially available HBV DNA quantitation assays [14]. In the present study, we aimed to assess the clinical performance of the Aptima assay using HBV reference panels and clinical samples. Aptima's performance was compared with the Roche COBAS® TaqMan® HBV assay using the generic High Pure System (HPS/CTM; Roche Diagnostics GmbH, Mannheim, Germany), a semiautomated assay that has demonstrated similar performance to other Roche HBV quantitative assay formats [11] as well as assays from other manufacturers [4, 15]. Performance characteristics such as analytical sensitivity, precision, reproducibility, linearity, bias and influence of the HBV genotype were evaluated.

Materials and methods

Retrospective clinical samples

Frozen, unlinked, surplus plasma or serum specimens from HBV-infected individuals were tested in parallel with the Aptima and HPS/CTM assays. Samples comprised the patient population of the laboratory and were selected based on historical HPS/CTM viral load results so as to span the dynamic range of the assays, and based on volume requirements and number of previous freeze-thaw cycles. To span the dynamic range of the assays, 20 HBV-negative samples, 20 HBV weak positive but non quantitated samples and ~20 samples at each log IU/mL from 1 to 8 log IU/mL were included. Low-level viremia samples (in particular in the range of 10–100 and 101–1000 IU/mL) were derived from patient samples diluted in negative plasma if needed. Samples were required to have a minimum volume of at least 1.5 mL in order to test in singlet with each assay (0.70 mL for Aptima and 0.5 mL for

HPS/CTM). Samples also had to be subjected to ≤3 freeze/thaw cycles. Aptima samples were tested in secondary tubes (SAT).

The study was performed in accordance with the requirements of the Ethical Board of the Local State Board of Physicians (Stuttgart, Germany) and was conducted in adherence to the Declaration of Helsinki. Only leftover samples from samples originally sent to our laboratory for routine HBV testing were used. All samples were anonymized using a unique identification number.

Reference panels

One commercially available panel and one external quality assessment (EQA) reference panel were used to test the assays for bias. The commercial panel was the Qnostics HBV Evaluation Panel (QNCM14-038-HBV; Qnostics, Ltd., Scotland), containing a seven-member panel of HBV genotypes A and D (at 1.7, 2.7 and 3.7 log IU/mL) and one HBV-negative member. The panel members were tested twice with Aptima and twice with HPS/CTM, and Aptima average results were compared with the target values given by the manufacturer and with HPS/CTM results. The EQA panel was the HBV Worldwide AccuSet™ Performance Panel (SeraCare Life Sciences, Milford, MA, USA), a seven-member panel containing seven distinct HBV genotypes (A, B, C, D, E, F and H) at viral loads ranging from 2.12 to 7.48 log IU/mL. Each EQA panel member was tested twice with Aptima. Average values obtained with Aptima were compared with data from three other assays (the Siemens VERSANT® HBV DNA 1.0 Assay (kPCR) [SVS], the Roche COBAS® AmpliPrep/COBAS® TaqMan® HBV Quantitative Test, v2.0 [CAP/CTM], and the Abbott RealTime HBV m2000 [ART] assays) reported by SeraCare [16], and to the consensus target values (average of the SVS, ART and CAP/CTM values). All three assays are commercially available real-time PCR assays for quantitative detection of HBV DNA in plasma and serum and have similar performance. Their linear range of quantitation is 1.1–8.8 log IU/mL for SVS, 1.3–9.2 log IU/mL for CAP/CTM and 1–9 log IU/mL for ART.

Linearity and precision

Linearity as well as bias was evaluated using the AcroMetrix™ HBV Panel (#950150; Thermofisher Scientific, Waltham, MA, USA; genotype unspecified), which was tested in duplicate with Aptima at given target concentrations of 50, 500, 5000, 50,000, 500,000, 5,000,000 and 50,000,000 IU/mL. Aptima and HPS/CTM linearity, bias and precision were compared using a well-characterized clinical sample representing genotype D, which was serially diluted with negative plasma to seven different target concentrations of 2, 3, 4, 5, 6 and 7 log IU/mL. Five replicates of each dilution level were tested side by side in both assays over 3 days.

Assay bias and precision at low viral load and influence of genotype

Three clinical samples with different genotypes (A, C and D) selected based on initial HPS/CTM viral load results were diluted with negative plasma to three different target concentrations each (1000, 100 and 25 IU/mL). Four replicates of each dilution level were tested in the Aptima and HPS/CTM assays over 3 days.

HBV quantitation assays

Aptima HBV Quant Dx assay: The Aptima assay is a fully automated real-time TMA assay that quantitates HBV genotypes A through H [17]. It is a dual-target assay with targets in the polymerase and surface genes, two highly conserved regions of HBV DNA. Aptima is designed to be run on the Panther system, a fully automated, random access platform. The assay's limit of detection (LOD) is 5.6 IU/mL for plasma and 4.3 IU/mL for serum, and its lower limit of quantification (LLOQ) is 10 IU/mL with a dynamic range of 1–9 log IU/mL. The test uses 0.5 mL of specimen volume per test, with an additional 0.2 mL for the dead volume.

COBAS® TaqMan® HBV test for use with the HPS: The HPS/CTM assay is a semiautomated, real-time PCR test that quantitates HBV genotypes A through G [18]. The assay is a single-target assay, targeting the precore/core region. HBV DNA is first manually extracted using the generic high pure Kit (Roche Diagnostics GmbH, Mannheim, Germany), which consists in lysis followed by nucleic acid extraction using glass beads. HBV DNA amplification by the PCR TaqMan assay is performed using the COBAS TaqMan 48 analyzer. The reported LOD is 5.9 IU/mL, the LLOQ is 29 IU/mL and the dynamic range of quantitation is 1.5 to 9 log IU/mL. The test uses 0.5 mL of specimen volume per test (no dead volume). The HPS/CTM assay is routinely used in the laboratory by operators who are trained and have demonstrated proficiency.

LiPA line probe assay: The LiPA assay (INNOGENETIC N.V., Gent, Belgium) identifies HBV genotypes A–H by detection of type-specific sequences in the HBV polymerase gene domain B–C. Biotinylated PCR products are hybridized to immobilized specific probes (reverse hybridization). After hybridization, conjugate and substrate are added, resulting in a colored precipitate. This test does not quantitate HBV DNA. The LiPA assay was used to determine the HBV genotypes in clinical samples if the sample volume was sufficient.

Data analyses

All samples tested in this study gave valid results with both the Aptima and the HPS/CTM assays and were included in the analysis. The agreement between assay results for HBV qualitative results (negative, detected but below the LLOQ [<LLOQ], quantitated and above the upper limit of quantitation [>ULQ]) was assessed by tabulation of paired data and by calculating the degree of agreement (κ) and the associated standard error (SE) and 95% confidence interval (CI). For clinical samples quantitated by both assays, agreement between assay values was assessed using the Deming regression analysis with calculation of the correlation coefficient (using Pearson's correlation) and the Bland-Altman analysis, which plots (for each sample) the difference between assay values against the average of the two assays. Assay linearity and bias was assessed by comparing assay results to consensus target concentrations using regression analysis and calculation of the correlation coefficient (Pearson's correlation). Assay precision was evaluated by calculating the standard deviation (SD) for the viral load values obtained in each assay for the replicates tested. All analyses were performed using GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA, USA).

Results

Detection and quantitation of HBV DNA in patients' samples

A total of 159 retrospective clinical samples were tested in parallel with Aptima and HPS/CTM (Table 1). The concordance between the assays' qualitative data was good with the number of observed agreements equal to 140/159 (88.1%) ($\kappa = 0.75$, SE = 0.05, 95% confidence interval [CI] = 0.651–0.845). Overall, we found 13 positive samples with viral loads below the LLOQ in both assays. Additionally, in seven samples with Aptima results positive but below the LLOQ, we found negative results in three cases and positive (>LLOQ) results in four cases with the HPS/CTM. Of the eight samples that tested positive but below the LLOQ in HPS/CTM, seven were negative and one positive (>LLOQ) in the Aptima. The four samples that were below the LLOQ in Aptima and quantitated by HPS/CTM had very low viral loads (9–15 IU/mL). This was true also for the sample that was below the LLOQ in HPS/CTM and quantitated by Aptima (23 IU/mL). Four samples that yielded values above the ULQ in HPS/CTM were quantitated in Aptima (at viral loads of 8.26–8.73 log IU/mL).

Of the 159 samples tested in both assays, 106 (66.7%) yielded quantitative values in both assays and were used for quantitative correlation analysis (Figure 1). The average viral loads ranged from 1.38 to 7.6 log IU/mL. The assays' quantitative values were highly correlated, with R = 0.92 and a slope and intercept of 0.86 (95% CI = 0.81–0.92) and 0.57 (95% CI = 0.38–0.76), respectively, in the Deming regression analysis (Figure 1A).

In the Bland-Altman analysis, 101/106 (95.3%) samples gave results within the 95% limit of agreement of the assays (−0.7812 to 0.9625 IU/mL); all five samples outside

Table 1: Correlation between Aptima and HPS/CTM qualitative results in 159 clinical samples.

HPS/CTM				Aptima	Total
	Negative	**<LLOQ**	**Quantitated**	**>ULQ**	
Negative	21	3	0	0	24
<LLOQ	7	13	1	0	21
Quantitated	0	4	106	0	110
>ULQ	0	0	4	0	4
Total	28	20	111	0	159

<LLOQ, below the lower limit of quantitation; >ULQ, above the upper limit of quantitation. Values represent the number of samples in each category. Agreement between the assays' qualitative results (in gray cells) is 140/159 = 88.05% (the percentage of agreement expected by chance is 52.62%). $\kappa = 0.75$; 95% CI = 0.651–0.845.
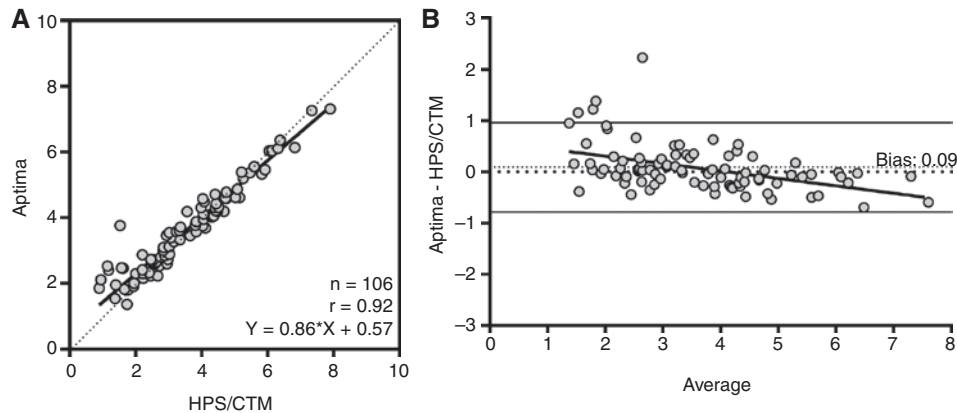
Figure 1: Correlation between Aptima and HPS/CTM quantitative results.
All values are in log IU/mL. (A) Deming regression analysis. The dotted line represents theoretical parity of results. (B) Bland-Altman analysis. Dotted line represents the overall bias (average difference between Aptima and HPS-CTM results across all samples). The horizontal solid lines represent the 95% limit of agreement −0.7812 to 0.9625. The diagonal solid line represents the difference between Aptima and HPS/CTM results in relation to VL values using regression analysis.

the 95% limit of agreement of the assays had average viral loads <2.65 log IU/mL and were quantitated at higher levels by Aptima (by 1.15–2.23 log IU/mL) (Figure 1B). Viral load values obtained with Aptima were on average higher than HPS/CTM values by only 0.09 (SD = 0.445) log IU/mL. However, we observed a trend in which samples with viral loads <4 log IU/mL had slightly higher values in Aptima, whereas those with viral loads >4 log IU/mL had higher values in HPS/CTM (Figure 1A and B).

Aptima performance with reference panels

Aptima testing of the Acrometrix HBV Evaluation Panel revealed an excellent agreement between Aptima data and target values as well as excellent linearity, with a Pearson's correlation coefficient R^2 of 0.9945 (Figure 2).

Aptima testing of the Accuset and Qnostic panels showed mean differences from the consensus target values ranging from 0.04 to 0.54 log IU/mL for Accuset and from 0.03 to 0.54 IU/mL for Qnostics (Table 2). The highest difference between Aptima data and the consensus values was observed for one genotype C sample (Accuset) and one genotype D sample (Qnostics) (difference of 0.54 IU/mL each). For the Qnostic panel, differences between Aptima and HPS/CTM results were all below 0.5 log IU/mL (range: 0.02–0.33 log IU/mL). For the Accuset panel, differences between Aptima and CAP/CTM were also all below 0.5 log IU/mL (range: 0.01–0.45 log IU/mL); differences between Aptima vs. ART and Aptima vs. SVS were below 0.5 log IU/mL, except for one sample (no. 24703; genotype C), which yielded differences slightly above 0.5 but below 0.7 log IU/mL.
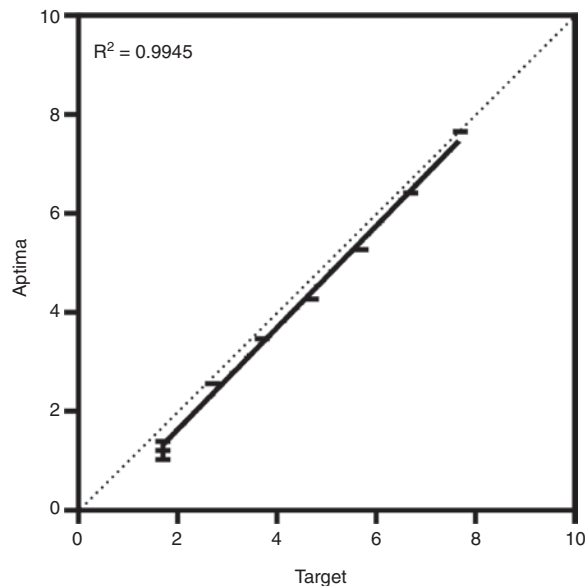

Figure 2: Aptima results with the Acrometrix panel.
n = 2 per target concentration level. Values are in log IU/mL. The dotted line represents theoretical parity of results.

Assay linearity and assay precision with clinical samples

Testing of a serially diluted clinical sample (genotype D) over the range of quantitation (1–7 log IU/mL) in Aptima and HPS/CTM assays showed an excellent agreement between assay results and target values. Both assays were linear, with slopes and intercepts equal to 0.99 and −0.09 for Aptima and 1.02 and 0.02 for HPS/CTM. The correlation coefficients vs. target were 0.9947 for Aptima and 0.9865

Table 2: Aptima and HPS/CTM results with the AccuSet and Qnostics HBV panels.

	Sample ID	GT	Target values				APT results			Difference between assay results			
			SVS	ART	CAP/CTM	Concensus	APT 1	APT 2	APT avg.	APT-SVS	APT-ART	APT-CAP/CTM	APT-CNS
AccuSet panel	24701	A	3.80	3.43	4.05	3.76	3.66	3.77	3.72	−0.08	0.29	−0.33	−0.04
	24702	B	3.31	3.26	3.30	3.29	2.86	2.83	2.85	−0.46	−0.41	−0.45	−0.44
	24703	C	5.22	5.13	5.02	5.12	4.64	4.51	4.58	−0.64	−0.55	−0.44	−0.54
	24704	D	3.43	3.13	3.36	3.31	3.32	3.38	3.35	−0.08	0.22	−0.01	0.04
	24705	E	7.25	7.77	7.41	7.48	7.44	7.44	7.44	0.19	−0.33	0.03	−0.04
	24706	F	5.24	5.33	4.98	5.19	4.91	4.96	4.93	−0.30	−0.40	−0.05	−0.25
	24707	H	0.00[a]	2.04	2.19	2.12	2.27	2.27	2.27	ND[a]	0.23	0.08	0.15

	Sample ID	GT	Target Consensus	APT results			HPS/CTM 1	HPS/CTM 2	HPS/CTM avg.	Difference between assay results	
				APT 1	APT 2	APT avg.				APT-CNS	APT-HPS/CTM
Qnostics Panel	1438A17	A	1.70	1.76	1.57	1.67	1.49	1.53	1.51	−0.03	0.16
	1438A27	A	2.70	2.58	2.54	2.56	2.49	2.65	2.58	−0.14	−0.02
	1438A27	A	2.70	2.61	2.56	2.59	2.46	2.54	2.50	−0.11	0.08
	1438A37	A	3.70	3.64	3.52	3.58	3.59	3.70	3.65	−0.12	−0.06
	1438D17	D	1.70	1.20	1.11	1.16	1.23	1.45	1.35	−0.54	−0.19
	1438D27	D	2.70	2.47	2.30	2.39	2.64	2.72	2.68	−0.31	−0.29
	1438D37	D	3.70	3.35	3.45	3.40	3.70	3.77	3.74	−0.30	−0.33

ID, identification; GT, genotype; APT, Aptima; SVS, Siemens Versant; ART, Abbott real time; avg., average; CNS, consensus; ND, not determined. All values are in log IU/mL. [a]This value (which is an outlier) was not taken into consideration in the calculation of the consensus or in calculation of the difference APT-SVS.
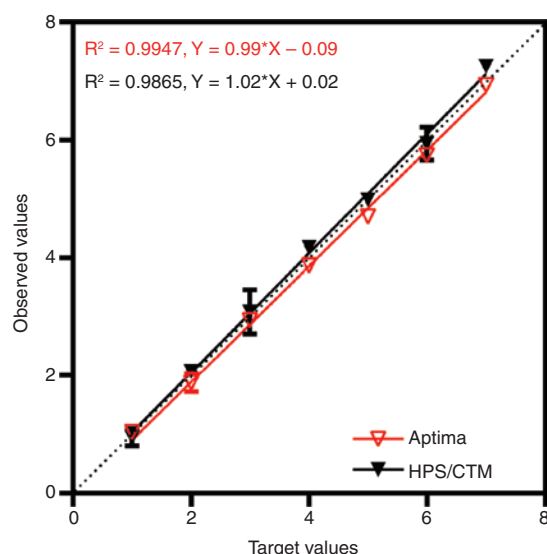
Target	Aptima			HPS/CTM		
	Mean	SD	n	Mean	SD	n
7.00	6.94	0.06	15	7.26	0.07	15
6.00	5.74	0.08	15	5.95	0.29	15
5.00	4.70	0.07	15	4.99	0.08	15
4.00	3.87	0.08	15	4.18	0.07	15
3.00	2.94	0.06	15	3.08	0.37	15
2.00	1.88	0.16	15	2.07	0.07	15
1.00	1.04	–	1	0.95	0.15	5

Figure 3: Assay linearity with a clinical sample.
Values are in log IU/mL. n = 15 per dilution level of a genotype D sample. The dotted line represents theoretical parity of observed and target values.

for HPS/CTM (Figure 3). Difference between assay results and target values were small, ranging from 0.04 to 0.3 log IU/mL for Aptima and 0.01–0.26 for HPS/CTM, demonstrating the low bias of the assays. For both assays, testing of each sample dilution in five replicates over 3 days (15 replicates per dilution) produced low SDs (0.06–0.16 for Aptima; 0.07–0.37 for HPS/CTM), demonstrating good overall assay precision, with Aptima showing greater precision compared with HPS/CTM.

Of note, at the 10 IU/mL level, the two assays were able to detect all replicates but only quantitated a small minority (1/15 for Aptima; 5/15 for HPS/CTM), likely because 10 IU/mL is close to the assays' LLOQ.

Bias and precision at low viral load and influence of genotype

Assay bias and precision at low viral loads was assessed by testing clinical samples of genotypes A, C and D at low viral loads (1000, 100 and 25 IU/mL) in triplicates over 4 days. Bias was low for both assays, with all assay values differing from the target by less than 0.5 IU/mL (0.04–0.32 for Aptima; 0.01–0.48 for HPS/CTM; Table 3). Although the genotype did not substantially impact assay bias, Aptima bias was slightly higher with genotype C, whereas HPS/CTM bias was slightly higher with genotype D. Precision was good for both assays, with SDs ranging from 0.06 to 0.20 for Aptima and from 0.05 to 0.28 for HPS/CTM across the viral loads. As expected, in most cases, assay precision

Table 3: Bias and precision of assay results at low viral loads and influence of genotype.

GT	Target[a]	Aptima			HPS/CTM		
		Mean	SD	n	Mean	SD	n
A	3.0	3.29	0.06	12	3.07	0.07	12
	2.0	2.32	0.09	12	1.98	0.08	12
	1.4	1.65	0.15	12	1.22	0.15	12
C	3.0	3.15	0.06	12	2.67	0.05	12
	2.0	2.22	0.09	12	1.52	0.28	12
	1.4	1.44	0.18	12	0.92	0.08	7
D	3.0	3.24	0.06	12	3.01	0.05	12
	2.0	2.33	0.06	12	2.01	0.06	12
	1.4	1.63	0.20	12	1.27	0.06	12

GT, genotype; SD, standard deviation. Values for target, mean and SD are in log IU/mL. Target VL based on initial HPS/CTM results.

decreased (SD increased) as the viral load decreased (for instance, for genotype A sample, Aptima SD was 0.06 at 1000 IU/mL, 0.09 at 100 IU/mL and 0.15 at 25 IU/mL). Aptima precision was similar with all genotypes, whereas HPS/CTM precision was best with genotype D. Overall, Aptima and HPS/CTM appeared to have similar bias and precision at low viral loads for the genotypes tested.

Discussion

In the present study, the Aptima assay demonstrated performance characteristics comparable with the HPS/CTM

assay with HBV reference panels and clinical samples, making the Aptima assay suitable for the quantitation of HBV DNA in clinical samples from HBV-infected patients undergoing treatment.

Comparison between Aptima and HPS/CTM in clinical samples showed (i) a high level of agreement (88.1%) between the assays qualitative data and (ii) a high correlation between the assays quantitative data (R = 0.92), with most (95.3%) of the samples falling within the 95% limit of agreement of the assays in the Bland-Altman analysis. Although the average difference between the assay was very small (0.09 log IU/mL), HPS/CTM tended to give higher values at high viral loads, whereas Aptima yielded higher values at low viral loads, with this change in bias occurring around 4 log IU/mL. This trend is opposite of that observed in the study comparing Aptima HIV Quant Dx assay to HPS/CTM HIV [19] and has not been seen when comparing Aptima HCV Quant Dx assay vs. HPS/CTM HCV [20]. Further, this trend was not observed in the linearity studies using diluted clinical samples where both assays had excellent linearity and correlation to target values throughout the dynamic range of the assays. The trend observed in the clinical sample correlation may be largely influenced by the five samples showing >1 log IU/mL difference as they all had low viral loads (below 1000 IU/mL). These discrepant samples would require further investigation that was not possible at the time of testing due to volume requirements; this may include testing by a third assay to resolve the discordance. Because virological breakthrough is defined as an increase in HBV DNA >1 log vs. the nadir, differences >1 log could impact therapeutic decisions, especially if they occur in low viral load samples. Thus, despite the small average difference between assay values (0.09 log IU/mL), it is recommended that HBV DNA monitoring for a given patient be performed with the same assay throughout the entire duration of treatment and that confirmatory testing be performed for samples that show large differences (>1 log) from the previous sample.

Testing of the Accuset and Qnostic HBV reference panels demonstrated that Aptima has excellent accuracy across all major HBV genotypes tested, except for a genotype C sample in the Accuset panel and a genotype D sample in the Qnostic panel for which Aptima showed a slight discrepancy (difference of 0.54 log each) with the consensus target concentration. For these two samples, however, no discrepancy between Aptima and HPS/CTM data was observed (assay differences were <0.5 log for all samples), echoing the comparable performance of the two assays. Aptima also demonstrated excellent correlation and linearity with the Acrometrix panel and with a serially diluted clinical sample (R^2 values >0.99).

Aptima had low bias and excellent precision/reproducibility over the entire range of quantitation (1–7 log IU/mL) as well as at low viral loads (25–1000 IU/mL) across the various genotype tested (A, C and D), with results similar to that of HPS/CTM. Both assays were able to quantitate all samples at 25 IU/mL, but only a minority of the samples at 10 IU/mL (most of which were detected but <LLOQ). Therefore, in this study, the two assays demonstrated similar performance at low viral loads, with an LOD <10 IU/mL and an LLOQ falling between 10 and 25 IU/mL. Although Aptima bias was slightly better with genotype C whereas HPS/CTM's bias was slightly better with genotypes A and D, overall, the two assays had similar overall bias and precision at low viral loads. Using an assay with high precision/reproducibility at low viral levels allows physicians to trust that changes in HBV DNA levels between two time points are true and not due to excessive assay variability, which is key to making appropriate therapeutic decisions in patients under treatment.

In conclusion, the Aptima HBV Quant Dx assay showed excellent overall performance in the detection and quantitation of HBV DNA in clinical samples, with comparable results with the well-established HPS/CTM assay. The Aptima assay offers the advantage of using a fully automated platform (the Panther system) with random access capability which, unlike the HPS/CTM assay, requires no manual operation. Full automation reduces time to result and improves workflow, which are highly desired assay characteristics for low to high-throughput laboratories.

References

1. World Health Organization. Media Center. Hepatitis B – Fact Sheet. Available at: http://www.who.int/mediacentre/factsheets/fs204/en/. Accessed: 12 Feb 2017.

2. European Association for the Study of the Liver (EASL). EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol 2012;57:167–85.

3. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH, et al. AASLD guidelines for treatment of chronic hepatitis B. Hepatology 2016;63:261–83.

4. Hochberger S, Althof D, Gallegos de Schrott R, Nachbaur N, Röck H, Leying H. Fully automated quantitation of hepatitis B virus (HBV) DNA in human plasma by the COBAS AmpliPrep/COBAS TaqMan system. J Clin Virol 2006;35:373–80.

5. Thibault V, Pichoud C, Mullen C, Rhoads J, Smith JB, Bitbol A, et al. Characterization of a new sensitive PCR assay for quantification of viral DNA isolated from patients with hepatitis B virus infections. J Clin Microbiol 2007;45:3948–53.

6. Garbuglia AR, Angeletti C, Lauria FN, Zaccaro P, Cocca AM, Pisciotta M, et al. Comparison of Versant HBV DNA 3.0 and COBAS AmpliPrep-COBAS TaqMan assays for hepatitis B DNA quantitation: possible clinical implications. J Virol Methods 2007;146:274–80.

7. Ciotti M, Marcuccilli F, Guenci T, Prignano MG, Perno CF. Evaluation of the Abbott RealTime HBV DNA assay and comparison to the Cobas AmpliPrep/Cobas TaqMan 48 assay in monitoring patients with chronic cases of hepatitis B. J Clin Microbiol 2008;46:1517–9.

8. Berger A, Gohl P, Stürmer M, Rabenau HF, Nauck M, Doerr HW. Detection and quantitation of HBV DNA in miniaturized samples: multi centre study to evaluate the performance of the COBAS® AmpliPrep/COBAS® TaqMan® hepatitis B virus (HBV) test v2.0 by the use of plasma or serum specimens. J Virol Methods 2010;169:404–8.

9. Chevaliez S, Bouvier-Alias M, Laperche S, Hézode C, Pawlotsky JM. Performance of version 2.0 of the Cobas AmpliPrep/Cobas TaqMan real-time PCR assay for hepatitis B virus DNA quantification. J Clin Microbiol 2010;48:3641–7.

10. Caliendo AM, Valsamakis A, Bremer JW, Ferreira-Gonzalez A, Granger S, Sabatini L, et al. Multilaboratory evaluation of real-time PCR tests for hepatitis B virus DNA quantification. J Clin Microbiol 2011;49:2854–8.

11. Pyne MT, Vest L, Clement J, Lee J, Rosvall JR, Luk K, et al. Comparison of three Roche hepatitis B virus viral load assay formats. J Clin Microbiol 2012;50:2337–42.

12. Morris CJ, Hill M, de Medina M, Herman C, Cloherty GA, Martin P. Comparison of detection and quantification of HBV DNA in chronic HBeAg negative and positive patients by Abbott Real-Time HBV and Roche Cobas TaqMan HBV assays. J Virol Methods 2013;193:391–3.

13. Yeh ML, Huang CF, Hsieh MY, Huang JF, Dai CY, Yu ML, et al. Comparison of the Abbott RealTime HBV assay with the Roche Cobas AmpliPrep/Cobas TaqMan HBV assay for HBV DNA detection and quantification. J Clin Virol 2014;60:206–14.

14. Chevaliez S, Dauvillier C, Dubernet F, Poveda JD, Laperche S, Hézode C, et al. The new Aptima HBV Quant Real-time TMA assay accurately quantifies hepatitis B virus DNA from genotypes A to F. J Clin Microbiol 2017;55:1211–9.

15. Weiss J, Wu H, Farrenkopf B, Schultz T, Song G, Shah S, et al. Real time TaqMan PCR detection and quantitation of HBV genotypes A-G with the use of an internal quantitation standard. Weiss J Clin Virol 2004;30:86–93.

16. SeraCare Life Sciences. HBV Worldwide AccuSetTM Performance Panel 0805-0247 – DATA SHEET. February 2015.

17. Hologic Inc. Aptima™ HBV Quant Assay [Package Insert]. 2015–11. Available at: http://www.hologic.com/sites/default/files/package%20inserts/hbv_pi.pdf. Accessed: 11 Jul 2016.

18. Roche Molecular Systems, Inc. COBAS® TaqMan® HBV Test for Use with the High Pure System. 8/2008.

19. Schalasta G, Börner A, Speicher A, Enders M. Comparative evaluation of the Aptima HIV-1 Quant Dx assay and COBAS TaqMan HIV-1 v2.0 assay using the Roche High Pure System for the quantification of HIV-1 RNA in plasma. Clin Chem Lab Med 2016;54:493–9.

20. Schalasta G, Speicher A, Börner A, Enders M. Performance of the new Aptima HCV Quant Dx assay in comparison to the Cobas TaqMan HCV2 test for use with the high pure system in detection and quantification of hepatitis C virus RNA in plasma or serum. J Clin Microbiol 2016;54:1101–7.