Journal of Clinical Virology 104 (2018) 83–88

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Analytical performance of the Hologic Aptima HBV Quant Assay and the COBAS Ampliprep/COBAS TaqMan HBV test v2.0 for the quantification of HBV DNA in plasma samples

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ARTICLE INFO

Keywords:
Viral load
HBV genotype
TMA
TaqMan
Linearity
Precision

ABSTRACT

Background: Quantification of HBV DNA is used for initiating and monitoring antiviral treatment. Analytical test performance consequently impacts treatment decisions.
Objectives: To compare the analytical performance of the Aptima HBV Quant Assay (Aptima) and the COBAS Ampliprep/COBAS TaqMan HBV Test v2.0 (CAPCTMv2) for the quantification of HBV DNA in plasma samples.
Study design: The performance of the two tests was compared on 129 prospective plasma samples, and on 63 archived plasma samples of which 53 were genotyped. Linearity of the two assays was assessed on dilutions series of three clinical samples (Genotype B, C, and D).
Results: Bland-Altman analysis of 120 clinical samples, which quantified in both tests, showed an average quantification bias (Aptima – CAPCTMv2) of −0.19 Log IU/mL (SD: 0.33 Log IU/mL). A single sample quantified more than three standard deviations higher in Aptima than in CAPCTMv2. Only minor differences were observed between genotype A (N = 4; average difference −0.01 Log IU/mL), B (N = 8; −0.13 Log IU/mL), C (N = 8; −0.31 Log IU/mL), D (N = 25; −0.22 Log IU/mL), and E (N = 7; −0.03 Log IU/mL). Deming regression showed that the two tests were excellently correlated (slope of the regression line 1.03; 95% CI: 0.998-1.068). Linearity of the tests was evaluated on dilution series and showed an excellent correlation of the two tests. Both tests were precise with %CV less than 3% for HBV DNA ≥3 Log IU/mL.
Conclusions: The Aptima and CAPCTMv2 tests are highly correlated, and both tests are useful for monitoring patients chronically infected with HBV.

1. Background

Hepatitis B virus (HBV) may cause chronic hepatitis B virus infection (CHB). The occurrence of CHB is dependent on the age of the individual acquiring infection [1]. The majority of infected adults achieves immune control of the infection and does not possess markers of active virus infection. In contrast, infection acquired perinatally or in early childhood often establishes CHB. CHB is associated with significant risk for development of liver failure and hepatocellular carcinoma and the risk for these complications is strongly associated with the levels of HBV DNA present in serum or plasma [2,3]. Antiviral treatment with nucleotide or nucleoside analogues is capable of suppressing viral replication and improve prognosis of chronic HBV infection [4–6]. The measurement of HBV DNA by sensitive and dynamic nucleic acid amplification technologies (NAAT) is therefore in addition to their use as diagnostic tools recommended as clinical support for the initiation and efficacy monitoring of antiviral therapy [7].

HBV is a reverse transcribing DNA virus, and because of the lack of proofreading activity of the reverse transcriptase nucleotide substitutions are continuously introduced into the virus genome [8]. HBV strains may on the basis of genome sequence analysis be classified into 10 genotypes (A–J) that are up to 8% divergent based on nucleotide alignment [9]. Sequence diversity constitutes a challenge for NAATs that for their analytical performance rely on the identification of oligonucleotide sequences uniformly conserved among clinical strains. The Hologic Aptima HBV Quant Assay is a relatively new test for the determination of HBV DNA in serum and plasma samples. It differs from other commercially available test by using target enrichment for nucleic acid purification and using real time transcription mediated amplification (TMA) instead of PCR for nucleic acid amplification and

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https://doi.org/10.1016/j.jcv.2018.05.007
Received 8 November 2017; Received in revised form 18 April 2018; Accepted 14 May 2018

quantification. Furthermore, it incorporates dual targets to improve homogenous quantification of diverse clinical HBV strains. In the present study, we compared the analytical performance of the new Aptima assay with the COBAS Ampliprep/COBAS TaqMan HBV version2.0 Test frequently used in many clinical laboratories.

2. Objectives

To compare the analytical performance of the Hologic Aptima HBV Quant Assay and the COBAS Ampliprep/COBAS TaqMan HBV DNA Test v2.0 for the quantification of HBV DNA in plasma samples.

3. Study design

3.1. HBV DNA determination

3.1.1. Aptima HBV quant assay

The Aptima assay was performed as described by the manufacturer. The test requires 700 µL of plasma and processes 500 µL. HBV viral nucleic acid is enriched by target capture using HBV specific oligonucleotides coupled to magnetic beads. The assay uses TMA to amplify two conserved regions within the S and P genes of HBV. Detection of the amplicon is done using oligonucleotide torches. The system is calibrated using triplicate determinations of a calibrator and verified using a high positive, low positive, and a negative control. Individual analyses are further verified using an internal control. The lower limit of quantification (LLOQ) is 10 IU/mL and the upper limit of quantification (ULOQ) is 9 Log IU/mL. The limit of detection (LOD) determined by 95% Probit analysis is reported by the manufacturer in plasma specimens to be 5.58 IU/mL based on the 3rd WHO International standard (NIBSC 10/264).

3.1.2. CAPCTMv2 HBV test

The CAPCTMv2 test was performed as described by the manufacturer. The test requires a sample volume of 650 µL and processes 500 µL on the AmpliPrep platform. The test targets the pre-Core/Core region of the HBV genome. It incorporates UNGase to prevent amplicon carry-over. Quantification is done relative to an internal, competitive quantification standard that is added to the sample along with lysis reagent. LLOQ of the test is 20 IU/mL and ULOQ is 8.23 Log IU/mL. LOD determined by 95% Probit analysis is reported to be 9 IU/mL based on the 2nd WHO International Standard (NIBSC 97/746).

3.2. Clinical specimens

Two set of specimens were used to evaluate the performance of the two tests.

The first set consisted of 63 residual plasma samples that had previously been tested in the CAPCTMv2 test and were compiled to span HBV DNA concentrations > LLOQ of the test (from below 2 Log IU/mL to above 8 Log IU/mL). Samples had been stored at −20 °C for up to 6 months and were assayed again in the two tests on the same day. Genotype was successfully determined in 53 of these samples. To this end, primers X02763_F248: 5′-CTAGACTCGTGGTGGACTT-3′ and X02763_R1272: AGTATGGATCGGCAGAGGAG were used to amplify a 1025 bp fragment of the P gene of HBV. Sequencing of the resulting PCR amplicons using the same primers as for amplification was done by Macrogen Europe (Amsterdam, the Netherlands).

The second set of specimens consisted of 129 prospectively collected residual plasma samples that were analyzed in the two tests without prior knowledge of their viral load. All samples were de-identified and individual samples were analyzed in the two tests on the same day.

3.3. Evaluation of linearity, diluted samples close to the LOD, and intraassay precision

Residual plasma from genotyped samples of HBV genotype B, C, and D were adjusted to approximately 6 or 7 Log IU/mL in the Aptima assay using HBV DNA-negative plasma (Nucleic Acid Test dilution matrix; Acrometrix). From this, dilution series were made using 4 tenfold dilution steps, then a further fourfold dilution step, and finally, a further four twofold dilution steps. Each dilution step was aliquoted to enable testing of each step in 6 replicates in the Aptima assay and at least 5 replicates in the CAPCTMv2 test. As a control, 6 replicates of HBV DNA negative plasma were tested in both tests for each dilution series.

4. Results

4.1. Evaluation of correlation between tests using clinical samples

The correlation between the two tests were evaluated on two specimen sets: 63 archived samples, which were compiled to include HBV DNA concentrations within the entire quantification range of the CAPCTMv2 test, and 129 consecutive clinical samples, which were included without prior knowledge of HBV DNA concentration. Of these 192 samples, 120 quantified within the linear measuring range of both tests and the results were analyzed in Bland-Altman analysis (Fig. 1). Average bias between the two tests was −0.19 Log IU/mL (Aptima – CAPCTMv2) with no obvious trend for increasing HBV concentration. For three samples, Aptima quantified more than two standard deviations and for one sample more than three standard deviations higher than CAPCTMv2. For three of these samples, there was sufficient residual sample to allow genotyping. The most deviant sample was genotype E, and the other two were genotype A and genotype D. Two samples quantified more than two standard deviations higher in CAPCTMv2 than in Aptima. No residual material was kept for the two samples, and hence, they were not genotyped.

Deming regression on the two sets of specimens did not yield significantly different regression estimates. Accordingly, the two data sets were combined. Deming regression of 120 samples that quantified within the linear range of both tests showed excellent correlation with a slope of the regression line of 1.033 (95% CI: 0.998-1.068) and with a Pearson correlation coefficient *r* of 0.984 (Fig. 2).

HBV genotyping was done on residual material in the set of 63 archived specimens and was successful on 53 samples. These were genotype A (N = 4), genotype B (N = 8), genotype C (N = 9), genotype D

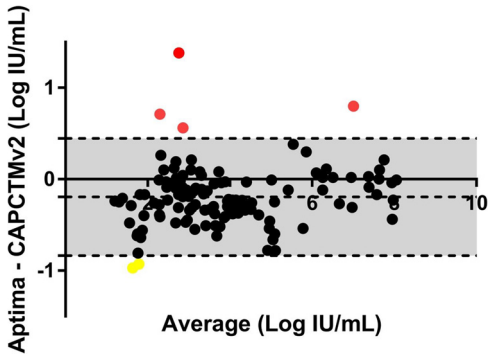

Fig. 1. Bland-Altman analysis.
Bland-Altman analysis of 120 clinical samples that quantified within the linear measuring range of both the Aptima assay and the CAPCTMv2 test. Average bias (−0.195 Log IU/mL; SD = 0.327 IU/mL) and the 95% confidence interval of the difference between the two test are shown by dotted lines and indicated by the shaded area. Six samples were outside the 95% confidence interval. Four samples, which Aptima quantified higher than CAPCTMv2, are indicated in red symbols, and two samples, which CAPCTMv2 quantified higher, are indicated in yellow symbols.