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Analytical and clinical performance of the Hologic Aptima HCV Quant Dx Assay for the quantification of HCV RNA in plasma samples

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ABSTRACT

Background: Chronic hepatitis C virus (HCV) infection can be effectively treated with directly acting antiviral (DAA) therapy. Measurement of HCV RNA is used to evaluate patient compliance and virological response during and after treatment.
Objectives: To compare the analytical performance of the Aptima HCV Quant Dx Assay (Aptima) and the COBAS Ampliprep/COBAS TaqMan HCV Test v2.0 (CAPCTMv2) for the quantification of HCV RNA in plasma samples, and compare the clinical utility of the two tests in patients undergoing treatment with DAA therapy.
Study design: Analytical performance was evaluated on two sets of plasma samples: 125 genotyped samples and 172 samples referred for quantification of HCV RNA. Furthermore, performance was evaluated using dilutions series of four samples containing HCV genotype 1a, 2b, 3a, and 4a, respectively. Clinical utility was evaluated on 118 plasma samples obtained from 13 patients undergoing treatment with DAAs.
Results: Deming regression of results from 187 plasma samples with HCV RNA > 2 Log IU/mL indicated that the Aptima assay quantified higher than the CAPCTMv2 test for HCV RNA > 4.9 Log IU/mL. The linearity of the Aptima assay was excellent across dilution series of four HCV genotypes (slope of the regression line: 1.00-1.02). The Aptima assay detected significantly more replicates below targeted 2 Log IU/mL than the CAPCTMv2 test, and yielded clearly interpretable results when used to analyze samples from patients treated with DAAs.
Conclusions: The analytical performance of the Aptima assay makes it well suited for monitoring patients with chronic HCV infection undergoing antiviral treatment.

1. Background

Determination of serum or plasma hepatitis C virus RNA (HCV RNA) is the most sensitive method for monitoring antiviral response and patient adherence during antiviral treatment of chronic HCV infection. Furthermore, the endpoint of antiviral therapy is sustained virological response (SVR) defined as an undetectable HCV RNA 12 or 24 weeks after end of treatment (EASL, 2017) (Swain et al., 2010) as determined by an analytically sensitive molecular method. Obviously, the analytical performance of the tests may impact clinical interpretation. Current commercially available tests include the COBAS TaqMan HCV v2.0 Test in combination with either the High Pure System (HPS/CTMv2) or the COBAS Ampliprep (CAPCTMv2) (Zitzer et al., 2013; Pas et al., 2013; Kessler et al., 2015), the Abbott RealTime HCV Assay (LaRue et al., 2012) (Vermehren et al., 2011) (Chevaliez et al., 2009), QIAGEN *artus* HCV QS-RGQ Test (Schønning, 2014), Siemens Versant HCV RNA 1.0 Assay (Gruner et al., 2015) (Kessler et al., 2013), and the VERIS MDx system HCV assay (Saune et al., 2016).

The Hologic Aptima HCV Dx Quant Assay (Aptima) is a relatively new assay, that employs target enrichment and real-time transcription mediated amplification (TMA). The assay is run on the fully automated Panther platform, and has recently been compared to the HPS/CTMv2 test (Schalasta et al., 2016), the Abbott RealTime HCV Assay (Garbuglia et al., 2017; Chevaliez et al., 2017; Worlock et al., 2017), and the C-APCTMv2 test (Chevaliez et al., 2017; Worlock et al., 2017). Here, we compared the APTIMA assay with the CAPCTMv2 test for both analytical performance on clinical plasma samples and for clinical utility on samples obtained from patients undergoing direct acting antiviral

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(DAA) therapy.

2. Objectives

To compare the analytical performance of the Aptima HCV Quant Dx Assay (Aptima) and the COBAS Ampliprep/COBAS TaqMan HCV Test v2.0 (CAPCTMv2) for the quantification of HCV RNA in plasma samples, and compare the clinical utility of the two tests in patients undergoing treatment with DAA therapy.

3. Study design

3.1. HCV RNA determination

3.1.1. Aptima HCV Quant Dx Assay

The Aptima assay was performed as described by the manufacturer (package insert). The test requires 700 µL of plasma and processes 500 µL. HCV RNA is enriched during purification through the use of HCV RNA specific oligonucleotides coupled to magnetic beads. The assay amplifies conserved regions of the HCV 5′-untranslated region (UTR) using TMA and detection 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. Furthermore, each assay includes an internal control. The lower limit of quantification (LLOQ) is 10 IU/mL and the limit of detection (LOD) (95% Probit) is reported by the manufacturer to be 4.3 IU/mL based on the 2nd. International WHO standard (NIBSC 96/798 genotype 1).

3.1.2. CAPCTMv2

The CAPCTMv2 test was performed as prescribed by the manufacturer (package insert). The test requires a sample volume of 650 µL and processes 500 µL on the AmpliPrep platform. The test incorporates UNGase to prevent amplicon carry-over and targets a conserved region of the HCV 5′-UTR. Quantification is done relative to an internal, competitive quantification standard that is added to the sample along with lysis reagent. LLOQ is reported to be 15 IU/mL and LOD (95% Probit) is 11 IU/mL based on the 3rd. International WHO Standard (NIBSC 06/100, genotype 1a).

3.2. Clinical specimens

Three sets of specimens were compared in the two tests. The first set consisted of 125 residual plasma specimens originally referred for HCV genotyping and stored at −20 °C. HCV-genotyping was done by sequencing of the Core/E1 and NS5 B regions as described previously (Clausen et al., 2011). HCV RNA signals deteriorate during prolonged storage at −20 °C. Therefore, the genotyped samples (N = 125) were thawed, and split into two aliquots to allow testing on the same day in the Aptima and CAPCTMv2 tests. Identical number of freeze/thaw cycles was maintained for the two aliquots. The second set consisted of 172 residual plasma samples referred for HCV RNA quantification. From this set of fresh samples, two aliquots were prepared and analyzed on the same day in the Aptima assay and CAPCTMv2 test, respectively. The third set consisted of 118 plasma samples from 13 patients (genotype 1a (3 patients), 1b (2 patients), 2b (1 patient), 3a (6 patients), and 4 (1 patient)) monitored during DAA treatment; the latter sample could not be assigned a subtype by sequencing. This set of samples was obtained from The Danish Database for Hepatitis B and C (DANHEP) biobank. DANHEP is a nationwide, clinical database for patients admitted to hospital with Chronic Hepatitis B and C infection (Hansen et al., 2009). All patients were treated in accordance with Danish guidelines. Samples, collected during therapy, were kept in the DANHEP biobank at −80 °C. All samples were analyzed in the Aptima assay, however, only 114/118 were analyzed in the CAPCTMv2 test due to insufficient specimen volume.

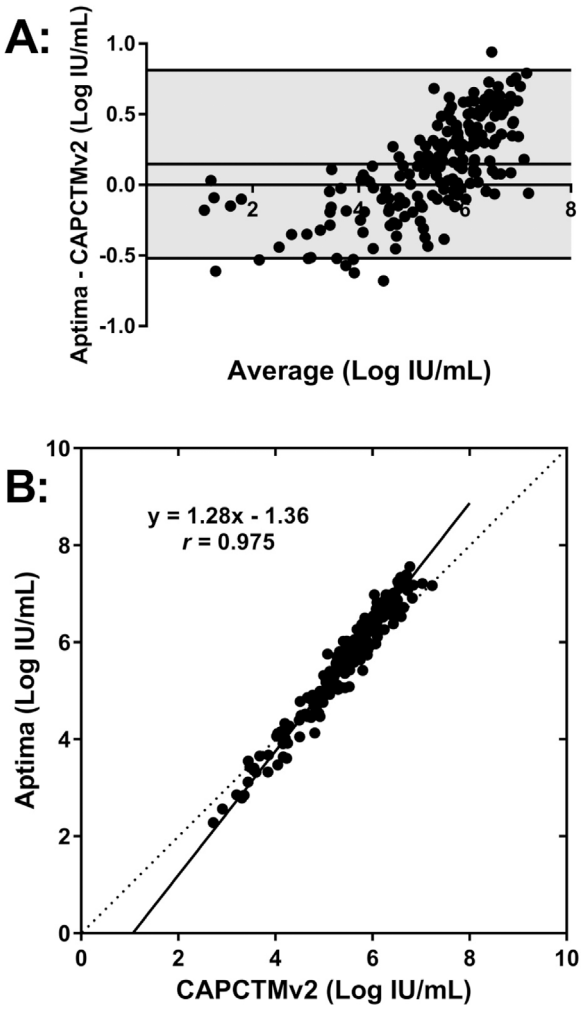

Fig. 1. Bland-Altman analysis and Deming regression on clinical samples.
A: Bland-Altman analysis of 194 plasma samples which were quantified in either test. Mean bias was 0.15 Log IU/mL (Aptima − CAPCTMv2). Mean bias +/− 1.96 standard deviations are also indicated. A clear trend for increasing difference with increasing HCV RNA was seen. At low HCV RNA, data converged toward the difference between the LLOQs of the two tests (-0.18 Log IU/mL). To avoid introduction of such bias in the regression estimates, 7 samples that quantified below 2 Log IU/mL in either test were omitted from Deming regression analysis. B: Deming regression of 187 plasma samples which yielded a HCV-RNA > 2 Log IU/mL in both the Aptima and CAPCTMv2 tests. The resulting equation of the regression line is shown as well as the Pearson correlation coefficient *r*. The unity line is indicated by the dashed line. The slope of the regression line (1.28, 95% CI 1.24-1.32) was significantly greater than 1 and the Aptima assay may be expected to quantify higher than the CAPCTMv2 test for HCV-RNA > 4.9 Log IU/mL.

3.3. Evaluation of linearity, limit of detection and intraassay precision

Residual plasma from genotyped samples of HCV genotype 1a, 2b, 3a, and 4a were adjusted to approximately 4 or 5 Log IU/mL in the Aptima assay using HCV-negative plasma (Nucleic Acid Test dilution matrix; Acrometrix). From this, dilution series were made using tenfold dilution steps in two or three steps to a concentration of approximately 2 Log IU/mL, and then further six two-fold dilutions steps were made. Each dilution step was aliquotted to enable testing of each step in 6 replicates in both tests. Additionally, for each dilution series HCV-negative plasma was tested in 6 replicates.

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