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Fully automated quantification of hepatitis C virus (HCV) RNA in human plasma and human serum by the COBAS® AmpliPrep/COBAS® TaqMan® System☆,☆☆

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Abstract

Background: HCV RNA is commonly recognized as key parameter for reliable diagnosis and treatment monitoring of HCV infection. Determination of blood HCV RNA concentrations reduces the pre-seroconversion period in the diagnosis of HCV infection and supports management of interferon alpha-based therapies of chronic HCV infection.
Objectives and study design: The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test combines automated extraction of nucleic acids on the COBAS® AmpliPrep Instrument with real-time PCR on the COBAS® TaqMan® Analyzer, thus greatly reducing hands-on time during sample preparation and amplification/detection. The test, which is calibrated to the 1st International HCV WHO Standard, was evaluated for sensitivity, dynamic range, precision, matrix equivalence, genotype inclusivity, interfering substances, diagnostic and analytical specificity, as well as for correlation with two other commercial tests for HCV RNA quantification.
Results: The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test demonstrated a >6-log dynamic range of 43–6.90E+7 IU/mL, a sensitivity (95% hit rate) of at least 15 IU/mL for HCV WHO Standard and a comparable quantification of genotypes 1–6. HCV quantification results were in good correlation with those obtained by the COBAS® AMPLICOR® HCV MONITOR Test v2.0 and the VERSANT® HCV RNA 3.0 test.
Conclusions: The fully automated COBAS® AmpliPrep/COBAS® TaqMan® HCV Test excellently accomplishes the requirements for highly sensitive detection and reliable quantification of HCV in clinical samples and thus improves therapy monitoring and management of HCV infection.

Keywords: HCV; Automated RNA extraction; Real-time PCR; Therapy monitoring

1. Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver disease, such as liver cancer and represents a common indication for liver transplantation (Hoofnagle, 2002). HCV has infected 170 million people worldwide and is responsible for 90–95% of the cases of post-transfusion non-A and non-B hepatitis as it can be transmitted by blood and blood products (Afdhal, 2004). Based on nucleotide sequence differences, six major HCV genotypes 1–6 have been described, which are further divided into subtypes. HCV genotypes show specific geographical distribution and association with particular risk groups for infection (Simmonds, 2004).

HCV RNA determination enables the diagnosis of active viremia prior to immunological seroconversion (Puoti et al., 1992) and was reported to have prognostic value for treatment outcomes of anti-viral therapy (Davis, 2002). High viral loads

Abbreviations: bDNA, VERSANT® HCV RNA 3.0 test; CV, coefficient of variation; HCV, hepatits C virus; LOD, limit of detection; NAT, nucleic acid amplification technique; PCR, polymerase chain reaction; RT, reverse transcriptase; QS, quantification standard; SD, standard deviation

☆ *Note*: This test is currently not available for sale in the United States.

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were associated with decreased rates of response to interferon therapy (Yamada et al., 1995) and a >2 log 10 titer decrease in HCV RNA concentrations during the early phase of treatment (2–12 weeks) was shown to predict effective treatment responses (Orito et al., 1995; Zeuzem et al., 1998). Therefore, accurate monitoring of HCV RNA concentrations is very important for treatment decisions, especially for treatment discontinuation in virologic nonresponders after 12 weeks of therapy (Berg et al., 2003; Chevaliez and Pawlotsky, 2006; NIH, 2002; Wong et al., 2003).

For HCV RNA determination quantitative tests based on target amplification (reverse transcriptase-polymerase chain reaction, RT-PCR) and signal amplification (branched DNA, bDNA) techniques with different sensitivity and linear measuring ranges are commercially available. The COBAS® AMPLICOR® HCV MONITOR Test v2.0 and the VERSANT® HCV RNA 3.0 test (bDNA) represent the PCR-based and the bDNA-based standard assays, respectively (Gerken et al., 2000; Ross et al., 2002). The COBAS® AMPLICOR® HCV MONITOR Test v2.0, however, requires dilutions for accurate quantification of high titer specimens (Gourlain et al., 2005). In addition, the COBAS® AMPLICOR® HCV MONITOR Test v2.0 and the bDNA assays display relatively low sensitivities of about 600 IU/mL and show limitations with respect to the lack of complete automation.

A fully automated test for HCV RNA quantification, the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, has recently become available. In the present study its performance is evaluated and compared to the COBAS® AMPLICOR® HCV MONITOR Test v2.0 and bDNA assays.

2. Technical system features and methods

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test (Roche Molecular Systems, Branchburg, NJ) combines automated isolation of nucleic acids on the COBAS® AmpliPrep Instrument with the automated amplification and detection on the COBAS® TaqMan® Analyzer. Reverse transcription and amplification primers as well as the probe are targeted to the HCV 5′-untranslated region of the HCV genome. For full process control a quantification standard (QS) is included in each sample which enables accurate titer quantification, compensating for effects of inhibition and controlling the preparation and amplification process. The procedure of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test is described in detail in the package insert (and in Sarrazin et al., 2006). Results are reported in International Units (IU/mL), traceable to the 1st WHO International Standard for Hepatitis C Virus RNA NAT Assay (NIBSC 96/790), genotype 1a (Saldanha et al., 1999).

The COBAS® AMPLICOR® HCV MONITOR Test v2.0 (Roche Molecular Systems, Branchburg, NJ, USA) is a semi-automated nucleic acid amplification assay, consisting of manual sample preparation and automated reverse transcription, amplification and detection steps on the COBAS® AMPLICOR® Analyzer (Gerken et al., 2000). According to the manufacturer, the linear range is 600–7.0E+05 IU/mL.

The VERSANT® HCV RNA 3.0 assay (bDNA, Bayer Diagnostics) is a signal amplification test based on branched DNA molecules with a reported linear range of 615–7.7E+06 IU/mL (Ross et al., 2002; Chevaliez and Pawlotsky, 2006).

All testing procedures were performed following the manufacturers instructions as described in the current package inserts.

3. Results

3.1. Sensitivity

The limit of detection (LOD) of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test was determined in EDTA–plasma and serum matrices with seven different concentration levels of the 1st International HCV WHO Standard RNA (Tables 1 and 2). Analytical sensitivites of 13.9 and

Table 1
Limit of detection of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test using the 1st WHO International Standard for Hepatitis C Virus RNA NAT Assay, NIBSC Code 96/790, diluted in EDTA plasma matrix

Nominal input (HCV RNA IU/mL)	No. of replicates	No. of positives	Positivity rate (%)
50.0	57	57	100
25.0	56	56	100
15.0	58	58	100
10.0	60	53	88
7.5	59	45	76
5.0	58	41	71
2.5	57	30	53
PROBIT 95% hit rate	13.9 IU/mL (95% confidence limits of 11.0–19.8 IU/mL)		

Data combined from two kit lots obtained from corresponding experiments with the seven indicated concentration levels.

Table 2
Limit of detection of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test using the 1st WHO International Standard for Hepatitis C Virus RNA NAT Assay, NIBSC Code 96/790, diluted in serum matrix

Nominal input (HCV RNA IU/mL)	No. of replicates	No. of positives	Positivity rate (%)
50.0	60	60	100
25.0	58	58	100
15.0	60	60	100
10.0	59	57	97
7.5	60	51	85
5.0	59	43	73
2.5	60	37	62
PROBIT 95% hit rate	10.5 IU/mL (95% confidence limits of 8.4–14.8 IU/mL)		

Data combined from two kit lots obtained from corresponding experiments with the seven indicated concentration levels.

Table 3
Limit of detection of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test using clinical specimens diluted in serum matrix representing genotypes 1a, 1b, 2a/c, 2b, 3a, 4, 5a and 6a

Genotype	No. of replicates	PROBIT 95% hit rate (IU/mL)	>95% hit rate analysis (IU/mL)
1a	≥28	8.2	10.0 (97%)
1b	24	10.7	10.0 (96%)
2a/c	≥23	6.5	7.5 (100%)
2b	≥20	9.0	10.0 (96%)
3a	24	11.4	15.0 (100%)
4	≥21	6.8	7.5 (96%)
5a	24	15.8	10.0 (96%)
6a	≥23	9.6	15.0 (100%)

Clinical samples were diluted in serum matrix to generate eight concentration levels of 50, 25, 15, 10, 7.5, 5, 2.5 and 0 IU/mL. Analysis was performed with at least two independent dilution series for each genotype.

10.5 IU/mL for EDTA plasma and serum, respectively, were obtained by PROBIT analysis (95% hitrate). The 95% confidence intervals overlapped for plasma and serum indicating equal sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test for the two different matrices. Furthermore, ≥95% hit rates at 15 and at 10 IU/mL were achieved for EDTA plasma and serum. In addition, the LOD of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test was evaluated for dilutions of clinical specimens representing genotypes 1–6 (Table 3). These samples yielded PROBIT values at 95% hit rate in the range of 6.5–15.8 IU/mL, revealing excellent accordance with the sensitivity determined for the 1st International HCV WHO Standard RNA.

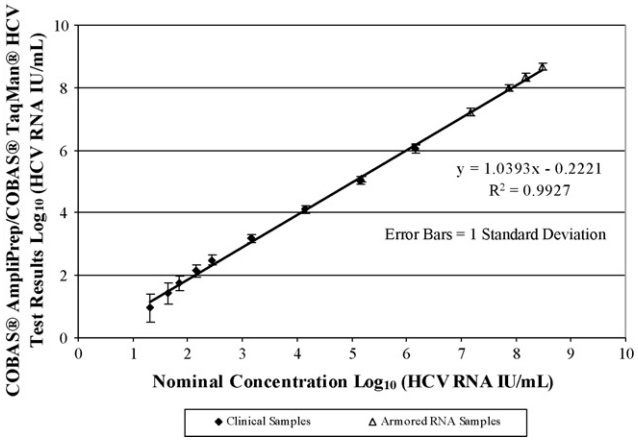

Fig. 1. Linearity of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test in EDTA plasma pool matrix: 15 nominal HCV RNA concentration levels (21, 43, 71, 142, 284, 1.42E+03, 1.42E+04, 1.42E+05, 1.42E+06 IU/mL, dilutions of a HCV RNA positive clinical sample, and 1.48E+05, 1.48E+06, 1.48E+07, 7.40E+07, 1.48E+08, 2.96E+08 IU/mL, dilutions of HCV Armored RNA) were tested in seven replicates on 16 days each. All concentration results and the nominal concentration for each level were $\log_{10}$ transformed. The $\log_{10}$ observed vs. $\log_{10}$ nominal were subjected to linear regression analysis. Outliers were not excluded from this analysis.

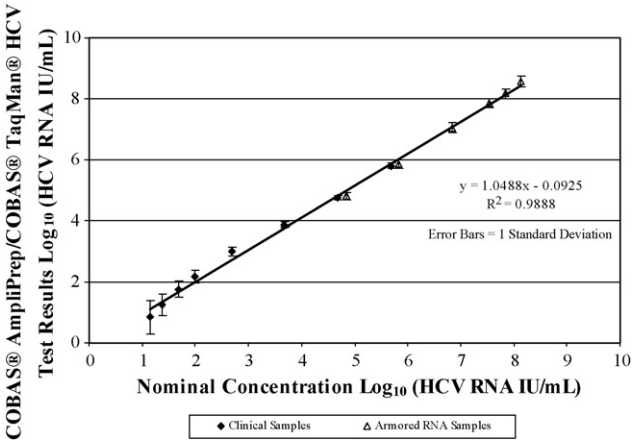

Fig. 2. Linearity for the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test in serum pool matrix: 14 nominal concentration levels (14, 24, 48, 96, 480, 4.80E+03, 4.80E+04, 4.80E+05 IU/mL, dilutions of the HCV positive clinical specimen, and 6.90E+04, 6.90E+05, 6.90E+06, 3.45E+07, 6.90E+07, 1.38E+08 IU/mL, dilutions of HCV Armored RNA) were tested in seven replicates on 16 days each. All concentration results and the nominal concentration for each level were $\log_{10}$ transformed. The $\log_{10}$ observed vs. $\log_{10}$ nominal were subjected to linear regression analysis. Outliers were not excluded from this analysis.

3.2. Linearity

The linearity and accuracy of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test was evaluated in EDTA plasma and serum by analyzing panels prepared from a high positive HCV RNA clinical specimen for the lower and middle part of the measuring range and additional panels based on Armored HCV RNA for the high end of the measuring range. The measuring range of the test is defined as the concentration range, for which all mean residual values are within the accuracy acceptance range of ± 0.3 $\log_{10}$ of the nominal concentrations. For EDTA plasma and serum measuring ranges of 43–2.96E+08 IU/mL (Fig. 1) and 14–6.90E+07 IU/mL (Fig. 2), respectively, were obtained in these experiments. Linear regression analyses of the quantitative results plotted as a function of the nominal concentration yielded coefficients of determination of $R^2 = 0.9927$ for EDTA plasma and $R^2 = 0.9888$ for serum, indicating the high degree of accuracy of the test. Combining the results for EDTA plasma and serum matrices, the overall linear measuring range of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test was defined as 43 ($\log_{10} = 1.63$) to 6.90E+07 ($\log_{10} = 7.84$) IU/mL.

3.3. Precision

The precision of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test across the measuring range was demonstrated for EDTA plasma matrix (Table 4) as well as for serum matrix (Table 5). Analysis was performed for three kit lots by testing seven replicates on at least 15 days for each concentration level. The standard deviations were ≤ 0.2 $\log_{10}$ for all concentrations levels and all three kit lots, indicat-

Table 4
Precision of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test for EDTA plasma samples

Input concentration (IU/mL)	Lot 1		Lot 2		Lot 3	
	Total %CV	Total SD in log	Total %CV	Total SD in log	Total %CV	Total SD in log
≥1.00E+02[a]	47	0.19	50	0.20	28	0.12
≥1.00E+03[a]	29	0.12	31	0.13	16	0.07
≥1.00E+04[a]	27	0.12	30	0.13	14	0.06
≥1.00E+05[b]	21	0.09	35	0.15	17	0.07
≥1.00E+06[b]	22	0.09	32	0.14	18	0.08
≥1.00E+07[b]	31	0.13	27	0.11	16	0.07

The percent coefficient of variation (%CV) was back-calculated from log-analysis for the results of each concentration level and the total standard deviation of log-transformed results was calculated.
[a] Clinical sample.
[b] Armored RNA.

Table 5
Precision of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test for serum samples

Input concentration (IU/mL)	Lot 1		Lot 2		Lot 3	
	Total %CV	Total SD in log	Total %CV	Total SD in log	Total %CV	Total SD in log
≥1.00E+02[a]	38	0.16	27	0.11	32	0.13
≥1.00E+03[a]	21	0.09	17	0.07	14	0.06
≥1.00E+04[a]	20	0.08	24	0.10	16	0.07
≥1.00E+05[b]	32	0.13	20	0.09	16	0.07
≥1.00E+06[b]	35	0.15	23	0.10	16	0.07
≥1.00E+07[b]	33	0.14	20	0.09	19	0.08

The percent coefficient of variation (%CV) was back-calculated from log-analysis for the results of each concentration level and the total standard deviation of log-transformed results was calculated.
[a] Clinical sample.
[b] Armored RNA.

ing high precision and lot to lot consistency of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test.

3.4. Matrix equivalence

Data from sensitivity, linearity and precision studies indicate equivalent HCV RNA quantification by the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test in EDTA plasma or serum. Analysis of matrix equivalence was extended to 29 matched clinical specimen pairs in EDTA plasma and in serum from 29 donors positive for HCV RNA. The results shown in Fig. 3 display that the $\log_{10}$ titer deviations between the two matrix types were lower than $\pm 0.3 \log_{10}$ for each of the individual sample pairs. The mean $\log_{10}$ deviation across all 29 tested sample pairs was 0.02, indicating excellent matrix equivalence for HCV RNA quantification in EDTA plasma and serum clinical samples by the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test.

3.5. Genotype inclusivity

The quantification of HCV genotypes by the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test was evaluated with the HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques, NIBSC Code 02/202, comprising panel members for HCV genotypes 1–6 (Saldanha and Heath, 2003). All results were within the accuracy acceptance range of $\pm 0.3 \log_{10}$ (Table 6). In addition, the quantification of clinical specimens of HCV genotypes 1a, 1b, 2a/c, 2b, 3a, 4 and 5 by the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test was assessed in comparison to the reference methods COBAS® AMPLICOR® HCV MONITOR Test v2.0 and bDNA (Table 7). The mean $\log_{10}$ deviations for COBAS® AmpliPrep/COBAS® TaqMan® HCV Test versus COBAS® AMPLICOR® HCV MONITOR Test v2.0 for genotypes 1a–3a displayed good agreement within the

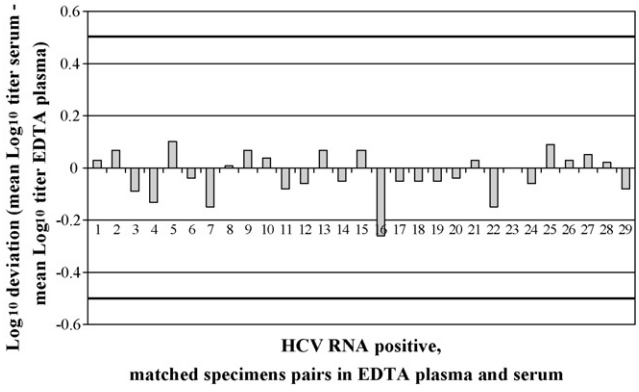

Fig. 3. Matrix equivalence of COBAS® AmpliPrep/COBAS® TaqMan® HCV Test: 29 HCV RNA positive, matched clinical specimen sets in EDTA plasma and serum (ProMedDx, USA) were analyzed in triplicate each. The mean $\log_{10}$ concentrations for each sample and the $\log_{10}$ deviation (mean $\log_{10}$ titer serum − mean $\log_{10}$ titer matched EDTA plasma) were calculated.

Table 6
COBAS® AmpliPrep/COBAS® TaqMan® HCV Test: genotype inclusivity for the NIBSC panel

Panel member	HCV genotype	Nominal $\log_{10}$ concentration (NIBSC)	$\log_{10}$ result COBAS® AmpliPrep/COBAS® TaqMan® HCV Test	$\log_{10}$ result COBAS® AmpliPrep/COBAS® TaqMan® HCV Test $-\log_{10}$ nominal (NIBSC)
NIBSC-1	1	3.0	3.2	0.2
NIBSC-2	2	3.0	3.3[a]	0.3
NIBSC-3	3	3.0	3.2	0.2
NIBSC-4	4	3.0	3.1	0.1
NIBSC-5	5	3.0	2.9	−0.1
NIBSC-6	6	3.0	3.3	0.3

The HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques, NIBSC Code 02/202, consisting of the genotypes 1–6 at concentrations of 1000 IU/mL was tested in single determination. For each panel member the $\log_{10}$ deviation of nominal and observed concentrations was calculated.
[a] Mean result of two replicates.

range of $\pm 0.2\log_{10}$. The mean $\log_{10}$ deviations for genotypes 4 and 5 were −0.5 and −0.4, respectively, yielding lower titers for the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test than for the COBAS® AMPLICOR® HCV MONITOR Test v2.0 test. Comparing bDNA versus COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, genotypes 2a/c and 5 showed equivalent results within $\pm 0.2\log_{10}$, whereas mean titers for 1a, 1b, 2b and 3a were lower and the mean titer for genotype 4 was higher for the bDNA assay. The comparison bDNA versus COBAS® AMPLICOR® HCV MONITOR Test v2.0 test displayed equivalent results within $\pm 0.2\log_{10}$ for genotypes 4 and 5, and lower bDNA results for genotypes 1a–3a.

3.6. Interferences

Elevated levels of triglycerides (up to 3186 mg/dL), bilirubin (up to 62 mg/dL), albumin (up to 10,200 mg/dL), hemoglobin (up to 470 mg/dL) and human DNA (up to 0.4 mg/dL) in specimens as well as the presence of markers for autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and antinuclear antibody were shown not to interfere with the quantification of HCV RNA by the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test. In addition, 27 drugs commonly used in the treatment of viral diseases (listed in Table 8), were tested at peak plasma level (C_{max}) and at three times the C_{max}, and did not display any interference with the quantification capability of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test.

3.7. Diagnostic specificity

The specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test was determined by analysis of 808 individual HCV RNA negative EDTA plasma specimens as well as of 768 serum specimens with two kit lots. For all specimens negative results for HCV RNA were obtained, yielding a specificity of 100% for the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test in this panel of blood donors (one sided lower 95% confidence limit: ≥99.6%).

3.8. Analytical specificity including exclusivity in non-HCV flaviviruses

The analytical specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test was evaluated by adding different pathogens (viruses including non-HCV flaviviruses, bacteria, yeast, see Table 9) or isolated cellular DNA (HTLV-II) to HCV negative human EDTA plasma or by analyzing specimens from infected patients. None of the non-HCV pathogens tested showed a positive result for the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test in HCV negative samples or interfered

Table 7
Comparison of genotype quantification by COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, COBAS® AMPLICOR HCV MONITOR Test v2.0 and VERSANT® HCV RNA 3.0 assay (bDNA)

HCV genotype	$\log_{10}$ result COBAS® AmpliPrep/COBAS® TaqMan® HCV Test − $\log_{10}$ result COBAS® AMPLICOR® HCV MONITOR Test v2.0		$\log_{10}$ result bDNA − $\log_{10}$ result COBAS® AmpliPrep/COBAS® TaqMan® HCV Test		$\log_{10}$ result bDNA − $\log_{10}$ result COBAS® AMPLICOR® HCV MONITOR Test v2.0	
	Number of specimens	Mean	Number of specimens	Mean	Number of specimens	Mean
1a	18	0.1	18	−0.5	18	−0.5
1b	29	0.2	26	−0.5	26	−0.5
2a/c	14	−0.2	14	−0.2	14	−0.5
2b	13	−0.2	12	−0.4	12	−0.6
3a	25	0.2	24	−0.7	24	−0.5
4	9	−0.5	9	0.5	9	−0.1
5	10	−0.4	10	0.0	10	−0.2

Clinical samples were either EDTA plasma or serum specimens (kindly provided by Dr. C. Sarrazin, Universitätsklinikum des Saarlandes, Homburg, Germany).

Table 8
Drug compounds tested for effects on quantification of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test

Nucleotide DNA polymerase inhibitors
Tenofovir
Adefovir dipivoxil
HIV protease inhibitors
Indinavir
Aquinavir
Ritonavir
Nelfinavir
Amprenavir
Lopinavir/ritonavir
Immune modulators
Interferon alpha-2a
Interferon alpha-2b
Peginterferon alpha-2a
Peginterferon alpha-2a + ribavirin
Interferon albpha-2b + ribavirin
Nucleoside reverse transcriptase and DNA polymerase inhibitors
Lamivudine
Zidovudine
Stavudine
Abacavir
Didanosine
Non-nucleoside HIV reverse transcriptase inhibitors
Nevirapine
Efavirenz
HIV fusion inhibitor
Enfuvirtide
Antidepressants
Paroxetine HCl
Fluoxetine
Sertraline
Compounds for the treatment of herpes viruses
Ganciclovir
Valganciclovir
Acyclovir

Table 9
List of specimens used for analytical specificity evaluation of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test

Non-HCV flaviviruses
West Nile virus
St. Louis encephalitis virus
Murray valley encephalitis virus
Dengue virus types 1–4
Yellow fever virus
Zika virus
Banzi virus
Ilheus virus
FSME virus
Hepatitis G virus (GBV-C)
Viruses
Adenovirus type 2
Cytomegalovirus
Epstein–Barr virus
Human herpes virus type 6
Herpes simplex virus type 1
Herpes simplex virus type 2
Human T-cell lymphotropic virus type 1
Human T-cell lymphotropic virus type 2
Influenza A
Hepatitis A virus
Hepatitis B virus
HIV-1B
Bacteria
Staphylococcus aureus
Propionibacterium acnes
Yeast
Candida albicans

with accurate quantification in HCV positive clinical samples.

3.9. Platform correlation

The capability of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test to accurately quantify HCV RNA in clinical samples was further evaluated in a method comparison using the COBAS® AMPLICOR® HCV MONITOR Test v2.0 and the bDNA assay as reference methods. In addition, two COBAS® AmpliPrep/COBAS® TaqMan® HCV Test kit lots were included in the analysis and yielded equivalent results (Table 10). The average deviation for each of the two COBAS® AmpliPrep/COBAS® TaqMan® HCV Test lots versus COBAS® AMPLICOR® HCV MONITOR Test v2.0 was 0.1 $\log_{10}$. The average deviation for each of the two COBAS® AmpliPrep/COBAS® TaqMan® HCV Test lots versus bDNA was −0.4 $\log_{10}$. The linear regression analysis of respective $\log_{10}$ concentration result pairs yielded coefficients of determination of $R^2 = 0.9525$ and 0.9149, for COBAS® AmpliPrep/COBAS® TaqMan® HCV Test versus COBAS® AMPLICOR® HCV MONITOR Test v2.0 (Fig. 4) and COBAS® AmpliPrep/COBAS® TaqMan® HCV Test versus bDNA (Fig. 5), respectively.

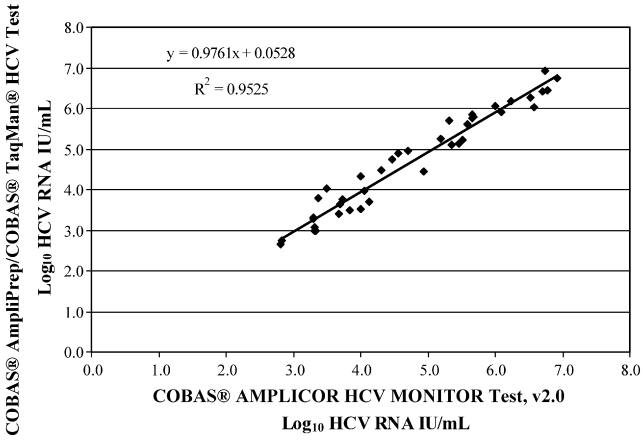

Fig. 4. Correlation of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test and the COBAS® AMPLICOR HCV MONITOR Test v2.0 on 40 plasma and serum specimens from HCV-infected patients.

Table 10
Platform correlation of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test

Methods compared	Mean $\log_{10}$ deviation
COBAS® AmpliPrep/COBAS® TaqMan® HCV Test Reagent Lot 1 vs. COBAS® AmpliPrep/COBAS® TaqMan® HCV Test Reagent Lot 2	0.0
COBAS® AmpliPrep/COBAS® TaqMan® HCV Test Reagent Lot 1 vs. COBAS® AMPLICOR HCM	0.1
COBAS® AmpliPrep/COBAS® TaqMan® HCV Test Reagent Lot 2 vs. COBAS® AMPLICOR HCM	0.1
COBAS® AmpliPrep/COBAS® TaqMan® HCV Test Reagent Lot 1 vs. VERSANT® HCV RNA	−0.4
COBAS® AmpliPrep/COBAS® TaqMan® HCV Test Reagent Lot 2 vs. VERSANT® HCV RNA	−0.4

Comparison Lot 1 vs. Lot 2 by analysis of 52 specimens (29 natural from HCV-infected patients (ProMedDx, Norton, MA), nine plasma and 20 serum, and 23 HCV spiked samples (for concentrations at the lower part of the dynamic range ≤1.0E+04 IU/mL), 18 plasma and five serum) in single determination. Comparison COBAS® AmpliPrep/COBAS® TaqMan® HCV Test vs. COBAS® AMPLICOR® HCV MONITOR Test v2.0 by analysis of 40 specimens (28 natural, nine plasma and 19 serum, and 12 spiked, seven plasma and five serum). Comparison COBAS® AmpliPrep/COBAS® TaqMan® HCV Test vs. bDNA by analysis of 34 specimens (26 natural, 17 plasma and nine serum, and eight spiked, four plasma and four serum). For data analysis, concentration results were $\log_{10}$ transformed. For method comparison the mean $\log_{10}$ deviations were calculated.

4. Discussion

The performance evaluation of the fully automated COBAS® AmpliPrep/COBAS® TaqMan® HCV Test presents a very robust and reliable assay displaying high sensitivity, specificity and accuracy for detection and quantification of HCV RNA genotypes 1–6 in EDTA plasma and serum matrices. The sensitivity of the assay is at least 15.0 IU/mL for the 1st International HCV WHO Standard (genotype 1a) and comparable for HCV genotypes 2–6. The greater than 6-$\log_{10}$ measuring range of 43–6.90E+7 IU/mL significantly extends the 3-$\log_{10}$ measuring range of the standard semi-automated COBAS® AMPLICOR® HCV MONITOR Test v2.0 Test (Gerken et al., 2000; Gourlain et al., 2005). The high sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test enables the quantification of specimens with very low viral loads ranging below the LOD of the COBAS® AMPLICOR® HCV MONITOR Test v2.0 and the bDNA assays (Gerken et al., 2000; Ross et al., 2002 Sarrazin et al., 2006). Due to accurate quantification of high titer specimens the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test could also be used to determine first phase viral kinetics up to 24 h after initiating treatment, a parameter for treatment monitoring which was suggested by Layden et al., 2002 to achieve earlier prediction of treatment responses.

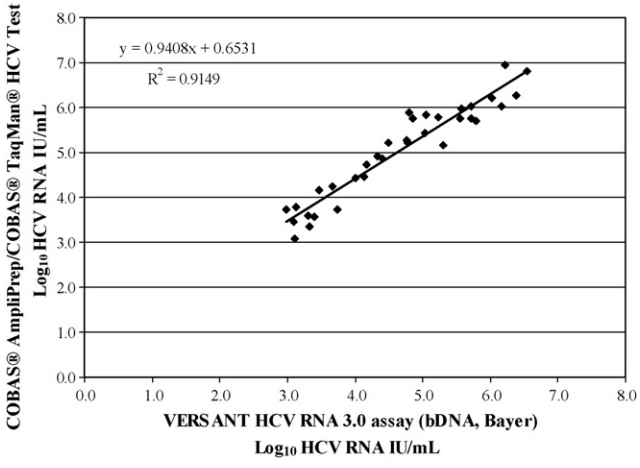

Fig. 5. Correlation of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test and the VERSANT® HCV RNA 3.0 assay on 34 plasma and serum specimens from HCV-infected patients.

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test accurately quantifies HCV genotypes 1–6 members of the HCV RNA Genotype Panel for NAT provided by NIBSC. Quantification of undiluted HCV genotype 1 through 5 clinical specimens by the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, compared with the standard reference method COBAS® AMPLICOR® HCV MONITOR Test v2.0, displayed high correlation for genotypes 1–3, whereas genotypes 4 and 5 yielded slightly lower titers within 0.5 log10 deviation for the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test. A slight underestimation of genotype 4 by the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test compared to the COBAS® AMPLICOR® HCV MONITOR Test v2.0 Test was recently also described by Sarrazin et al. (2006).

Comparing bDNA versus COBAS® AmpliPrep/COBAS® TaqMan® HCV Test and COBAS® AMPLICOR® HCV MONITOR Test v2.0, constant lower quantification results were obtained for genotypes 1–3 with the bDNA assay. In addition, the method comparison with clinical samples yielded lower quantitative results for the bDNA than for the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test and COBAS® AMPLICOR® HCV MONITOR Test v2.0 tests, indicating differences in the standardization of the bDNA and the two PCR-based assays. Due to these differences, it was recently recommended not to switch between different assays for clinical monitoring of HCV viral load during therapy, in order to ensure appropriate treatment decisions from HCV RNA quantification results (Sarrazin et al., 2006).

A major improvement compared to most other commercially available quantitative HCV assays is the complete automation provided by the COBAS® AmpliPrep/COBAS® TaqMan® system. The COBAS® AmpliPrep Instrument decreases the hands-on time for the sample preparation step by up to 76% compared to a manual method (Jungkind, 2001). The COBAS® AmpliPrep Instrument further automatically adds the eluate to the mastermix and transfers the tubes containing the PCR reaction mix to the COBAS® TaqMan®

Analyzer. This results in enhanced user convenience and a great reduction in labor requirements minimizing hands-on time. The automated procedure as well as real-time amplification and detection shorten the time to first result, automation decreases the risks of sample contaminations and ensures sample traceability throughout the entire procedure.

In conclusion, the fully automated COBAS® AmpliPrep/COBAS® TaqMan® HCV Test exhibits high sensitivity and specificity as well as a broad linear range for titer quantification of HCV genotypes 1–6 in serum and EDTA plasma thus meeting the high demands for monitoring disease progression in HCV infection as well as response to anti-viral treatment in the clinical routine.

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