Evaluation of performances of VERSANT HCV RNA 1.0 assay (kPCR) and Roche COBAS AmpliPrep/COBAS TaqMan HCV test v2.0 at low level viremia

Laura Mazzuti[1], Maria Antonietta Lozzi[2], Elisabetta Riva[3], Paola Maida[1], Francesca Falasca[1], Guido Antonelli[1], Ombretta Turriziani[1]

[1]Department of Molecular Medicine, Sapienza University of Rome, Italy;
[2]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Italy;
[3]University Campus Bio-Medico of Rome, Italy

SUMMARY

We assess the concordance between low level HCV values obtained using the VERSANT HCV RNA 1.0 Assay (kPCR) and COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test v2.0.
The correlation between the values obtained by the two RT-PCR assays for samples with quantifiable HCV RNA levels revealed that viral load measured by kPCR significantly correlated with that of the CAP/CTM (R=0.644, P<0.0001). The results show a good concordance (n=126/144, 87%); discordant results were mainly observed in the assessment of values below the lower limit of detection of the assays.
These variations may have an impact on clinical decisions for patients on HCV triple therapy or interferon-free regimens. It is therefore recommended to monitor individual patients with the same test throughout treatment.

Hepatitis C virus (HCV) infection is a significant global health problem, causing chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (Seeff, 2002). The overall prevalence of viral hepatitis worldwide is estimated to be 3% with over 175 million people infected with HCV and an estimated global incidence of three to four million new infections per year (de Oliveria Andrade *et al.*, 2009).

HCV cannot integrate into the host genome, and its persistence relies on continuous replication; thus, it can theoretically be eradicated from the host (Ippolito *et al.*, 2014; Ippolito *et al.*, 2015).

The primary goal of anti-HCV therapy is the achievement of a sustained virological response (SVR) characterized by undetectable HCV RNA (<10-15 IU/mL) 12 weeks or 24 weeks after the end of treatment: this corresponds to viral eradication in more than 99% of cases.

Until recently, combination therapy with peginterferon alfa/ ribavirin (P/R) had been the standard approach to the management of chronic HCV infection. HCV genotype 1 treatment options have evolved rapidly in recent years, with the introduction in clinical practice of direct-acting antivirals (DAAs), such as protease inhibitors, and NS5A and NS5B polymerase inhibitors, that have progressively improved efficacy and reduced therapy-induced side effects.

Key words:
HCV, Viral load, Real-time PCR, VERSANT HCV RNA, COBAS TaqMan HCV.

Corresponding author:
Ombretta Turriziani
E-mail: ombretta.turriziani@uniroma1.it

DAA-containing regimens offer higher SVR rates and in several cases shorter treatment durations, but management and monitoring of patients receiving antiviral therapy has become more complicated.

Only HCV RNA quantitative assays based on real-time (RT) polymerase chain reaction technology (limit of detection (LOD) below 15 IU/ml) are appropriate for managing patients treated with DAA regimens (Cobb *et al.*, 2013); qualitative results can also be reported. Specifically, values can be reported as target not detected (TND) or < LOD but detectable (TD). Since assessing rapid virological response can be used to guide treatment duration, the ability of an assay to accurately detect low levels of viremia is critical to careful treatment management.

The aim of our study was to assess the concordance of two real-time PCR-based assays when used to detect low level HCV viremia. Specifically, we compared the VERSANT HCV RNA 1.0 assay (kPCR) (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) and the COBAS AmpliPrep/ COBAS TaqMan HCV quantitative test v2.0 (CAP/CTM, Roche Molecular Systems, Pleasanton, CA, USA). To our knowledge, few publications are available comparing the results obtained by these assays for HCV samples with low level viremia.

A total of 33 serum samples were retrospectively selected from routine diagnostic specimens from patients with chronic HCV infection. An aliquot of each anonymized sample was analyzed using VERSANT HCV RNA 1.0 assay (kPCR) (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) and COBAS AmpliPrep/COBAS TaqMan HCV quantitative test v2.0 (CAP/CTM, Roche Molecular Systems, Pleasanton, CA, US). AS the low level of viremia was one of the prerequisites for the analysis, these samples were

diluted with pools of HCV RNA-negative human serum to prepare a dilution series containing 500, 100, 10, and 1 IU/mL, based on the Siemens assay. One hundred and thirty-two diluted samples (33 samples/four levels each) were aliquoted into sufficient aliquots to allow testing in triplicate on both platforms, and stored at -80°C until used. In addition, 12 samples from SVR patients were analyzed with the Siemens assay and the Roche assay in parallel.

Two commercially available quantitative HCV RNA assays were evaluated during this investigation: VERSANT HCV RNA 1.0 assay (kPCR) (VERSANT kPCR, Siemens Healthcare Diagnostics, Tarrytown, NY, USA) and COBAS AmpliPrep/COBAS TaqMan HCV quantitative test v2.0 (CAP/CTM, Roche Molecular Systems, Pleasanton, CA, USA). The dynamic ranges for the two methods are 15 to 10^8 IU/mL for VERSANT kPCR, and 15 to 5×10^7 for CAP/CTM.

The Roche system is comprised of the automated COBAS AmpliPrep sample preparation instrument connected to the COBAS TaqMan instrument designed for real-time PCR amplification and detection. The Siemens system includes the Sample Preparation (SP) Module designed for automated sample preparation and the Amplification/Detection (AD) Module designed for real-time PCR amplification and detection. Three replicates of each sample were tested in each assays. All testing procedures were performed according to the manufacturer's instructions as described in the respective package inserts. Runs were considered valid if all conditions outlined in the manufacturer's package insert instructions were fulfilled. For both assays, the LOD and the lower limit of quantification (LLOQ) are identical at 15 HCV RNA IU/ml.

Descriptive statistics are shown as the mean ± the standard deviation where appropriate. Relationships between quantitative variables were studied by means of regression analysis. Bland-Altman plots, in which the differences between the two techniques are plotted against the averages of the two techniques, were used for better visualization of differences between assay quantifications. Statistical anal-

yses were performed using MedCalc statistical software (MedCalc Software bvba, Ostend, Belgium), and *P* values less than 0.05 were considered significant. The overall concordance between CAP/ CTM and VERSANT kPCR was analyzed using samples tested in both assays. The results (mean of the three replicates) show a good degree of concordance (n=126/144, 87%). Of the 126 concordant samples, 9 had undetectable HCV RNA results by both assays whereas 117 yielded positive results. The majority of the 18 discordant samples (83%) had HCV RNA viral loads that were detected but below the limit of quantification of one of the two assays, but were not detected in the other assay. Specifically, 12 samples had detectable HCV-RNA below the lower limit of quantification (LLOQ; <15 IU/mL) by CAP/CTM but were not detected by VERSANT kPCR, and 3 were below the LLOQ in VERSANT kPCR but were not detected by CAP/CTM. Among the discordant samples, 1 was quantifiable by CAP/CTM and undetectable by VERSANT kPCR, and 2 samples were quantifiable by VERSANT kPCR and undetectable by CAP/CTM (*Table 1*). A total of 82 samples with HCV RNA values within the overlapping quantitation range of both assays were used to evaluate the overall quantitative correlation. Analysis of the correlation between the values obtained by the two

Table 1 - *Comparison of results from 142 samples obtained by the VERSANT HCV RNA 1.0 Assay (kPCR) and the COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test v2.0.*

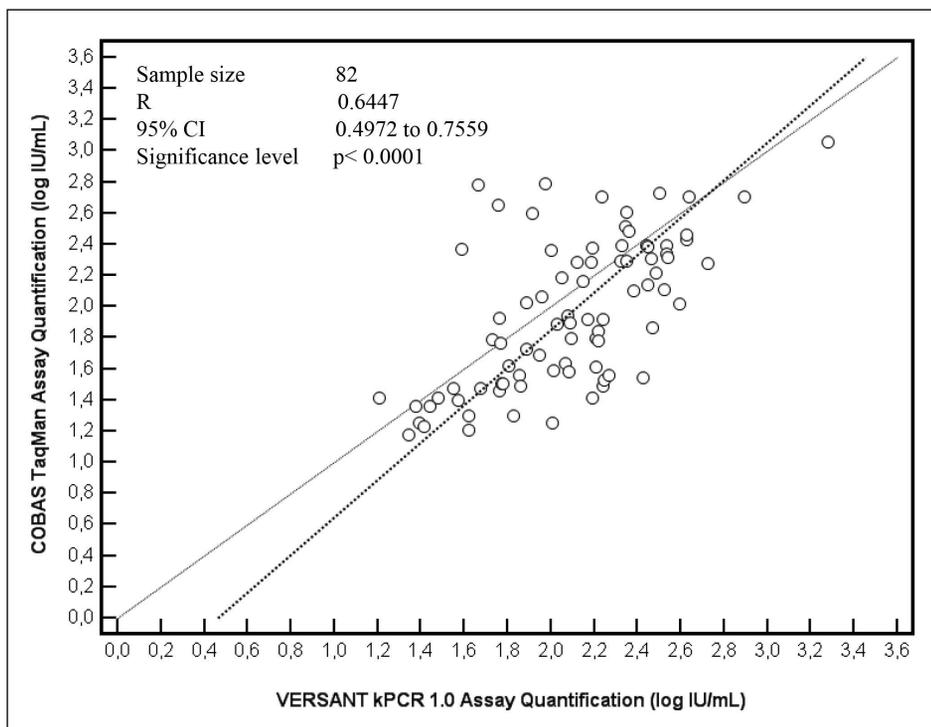|		Roche assay		
		Not detected	*<15 IU/mL, but detected*	*≤15 IU/ mL*
Siemens assay	Not detected	9	12	1
	<15 IU/mL, but detected	3	12	2
	≤15 IU/mL	2	19	82

Figure 1 - *Correlation of HCV-RNA quantification results between the VERSANT HCV RNA 1.0 assay (kPCR) and the COBAS AmpliPrep/COBAS TaqMan HCV quantitative test v2.0 among 82 specimens.*

Sample size	82
R	0.6447
95% CI	0.4972 to 0.7559
Significance level	p< 0.0001

real-time PCR assays revealed that HCV RNA viral load measured by VERSANT kPCR significantly correlated with that of the CAP/CTM (R=0.644, P<0.0001) (*Figure 1*). A Bland- Altman plot of the results for these samples was used to determine the agreement between the two assays. The differences between the two assays were plotted against the averaged $\log_{10}$ results. VERSANT kPCR and CAP/CTM showed good agreement for quantitative HCV RNA results with a mean difference of 0.13 log IU/mL (limit of agreement of -0.60 and 0.87 $\log_{10}$ IU/mL) (*Figure 2*).

Accurate HCV-RNA quantification is essential for managing the treatment of patients with chronic hepatitis C. This is particularly important when DAAs are used (Fried *et al.*, 2012; Kessler *et al.*, 2013). A more precise prediction of the individual relapse risk in chronic hepatitis C virus infection can be obtained by kinetics of minimal residual viremia at weeks 4, 8, and 12 in combination with levels of baseline viremia (Wiegand *et al.*, 2011). Currently the HCV-RNA level is assessed using real-time PCR-based assays, because of their specificity, sensitivity, accuracy and broad dynamic range. Both the AASLD (Ghany *et al.*, 2011; Pyne *et al.*, 2013) and EASL (European Association for the Study of the Liver, 2015) treatment guidelines recommend the use of a sensitive PCR assay with a lower limit of detection ≤15 IU/ml. Considering the potency of the new DAAs, it has become important to distinguish between detectable and undetectable HCV RNA for patients with viral loads below the lower limit of quantification. For instance, current AASLD and EASL guidelines include both recommendations for shortened treatment duration and futility rules that depend on patients obtaining undetectable HCV RNA (Jacobson *et al.*, 2011; Poordad *et al.*, 2011; Pawlotsky, 2014). Therefore the evaluation of viremia levels near the lower limit of detection can impact treatment decisions during therapy. For this reason, it is important to investigate the robustness of the assays to detect HCV RNA and the concordance between low level HCV values obtained using different real-time-PCR assays.

To date, few data are available on the implications of using different HCV RNA assays on treatment duration and outcome. In this study, two highly sensitive commercial assays for HCV RNA quantification, available in many countries, the VERSANT HCV RNA 1.0 assay (kPCR) (VERSANT kPCR, Siemens Healthcare Diagnostics, Tarrytown, NY, USA) and the COBAS AmpliPrep/COBAS TaqMan HCV Test v2.0 (CAP/CTM, Roche Molecular Systems, Pleasanton, CA, USA), were used to determine the HCV RNA in 144 serum samples with low level viremia.

A good correlation was found between the two assays in the ability to evaluate samples selected in a very low range of viremia, where it is known that such assay, may lose "precision"; in addition a concordance of 87% between the 2 tests was found. Discordant results were mainly observed in the assessment of values below LOD. These data are in agreement with a previous paper (Maasoumy *et al.*, 2014; Vermehren *et al.*, 2014), showing that repeated testing of samples during treatment by different assays led to both detectable and undetectable HCV RNA results. In our study only 3 samples (2%) showed discordant data, resulting undetectable by one of the assays and >15 IU/ml by the other assay.

Probably, measuring viral loads at levels near the limit of detection inherently increases the stochastic noise in sampling. Discrepancies between the two methods should be due to the total analytic error concept, which describes important and well-regarded approaches combining random error, systematic error, and operational specifications (Wiesmann *et al.*, 2014). These variations may have an impact on clinical decisions for patients on HCV triple therapy and may argue for assay-specific treatment decision points (equivalent to reference values).

In conclusion, only highly sensitive HCV RNA assays should be used to monitor viral kinetics and predict the risk of relapse. Specifically patients on DAA-based therapy should undergo close HCV RNA monitoring, ideally with the same HCV RNA assay in the same laboratory (Peiffer
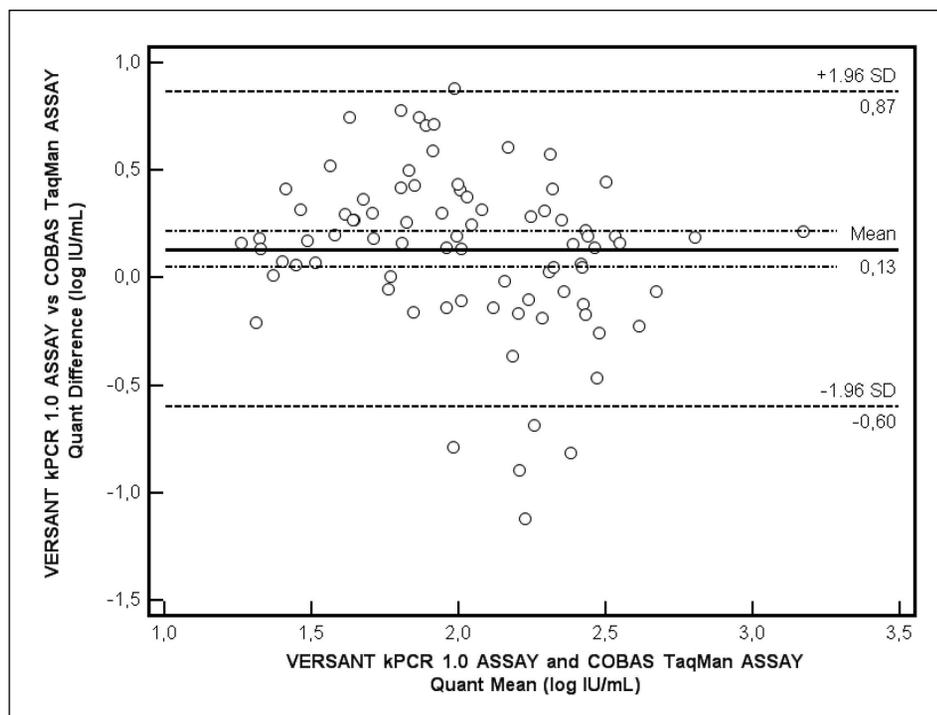

Figure 2 - *Bland-Altman plot analysis of HCV RNA levels determined by VERSANT HCV RNA 1.0 assay (kPCR) and the COBAS AmpliPrep/COBAS TaqMan HCV quantitative test v2.0. Data from 82 samples are shown. The middle dashed line represents the mean difference between the two assays. The outer dashed lines represent the limits of agreement (±1.96 standard deviation) and the fine line represents the reference line.*

& Sarrazin 2013). Indeed, clinicians should be aware that there could be analytical differences between viral load assays at low viremia levels. Therefore, the interpretation of Log RNA decline in patients with a starting HCV RNA viral load of less than 1000 IU/ml may be erroneous and lead to inappropriate therapeutic decisions in the context of stopping rules based on virologic response (Kessler *et al.*, 2013). Moreover, a comparison of quantification is recommended prior to switching assays during ongoing therapy (Wiesmann *et al.*, 2014).

Competing Interests Statement
All authors declare that they have no competing financial, professional or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

References

Cobb B., Pockros P.J., Vilchez R.A., Vierling J.M. (2013). HCV RNA viral load assessments in the era of direct-acting antivirals. *Am J Gastroenterol.* **108**, 471-475.

de Oliveria Andrade L.J., D'Oliveira A., Melo R.C., De Souza E.C., Costa Silva C.A., Paraná R. (2009). Association Between Hepatitis C and Hepatocellular Carcinoma. *J Glob Infect Dis.* **1**, 33-37.

European Association for the Study of the Liver; EASL Recommendations on Treatment of Hepatitis C 201. (2015). *J Hepatol.* **63**, 199-236.

Fried M.W. (2012). Maximizing opportunities and avoiding mistakes in triple therapy for hepatitis C virus. *Gastroenterology.* **142**, 1314-1323.e1.

Ghany M.G., Nelson D.R., Strader D.B., Thomas D.L., Seeff L.B. (2011). American Association for Study of Liver Diseases. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guidelines by the American Association for the Study of Liver Diseases. *Hepatology.* **54**, 1433-1444.

Ippolito G., Antonelli G., Capobianchi M.R., Lanini S. (2014) Hepatitis C Virus treatment revolution: needs for close monitoring. *Clin Microbiol Infect.* DOI: 10.1111/1469-0691.12693

Ippolito G., Capobianchi M.R., Lanini S., Antonelli G. (2015). Is hepatitis C virus eradication around the corner only 25 years after its discovery? *Int J Antimicrob Agents.* **45**, 111-112.

Jacobson I.M., McHutchison J.G., Dusheiko G., Di Bisciglie A.M., Reddy K.R., Bzowej N.H., Marcellin P., Muir A.J., Ferenci P., Flisiak R., George J., Rizzetto M., Shouval D., Sola R., Terg R.A., Yoshida E.M., Adda N., Bengtsson L., Sankoh A.J., Kieffer T.L., George S., Kauffman R.S., Zeuzem S., ADVANCE Study Team. (2011). Telaprevir for previously untreated chronic Hepatitis C virus infection. *N Engl J Med.* **364**, 2405-2416.

Kessler H.H., Hübner M., Konrad P.M., Stelzl E., Stübler M.M., Baser M.H., Santner B.I. (2013). Genotype impact on HCV RNA levels determined with the VERSANT HCV RNA 1.0 assay (kPCR). *J Clin Virol.* **58**, 522-527.

Maasoumy B., Cobb B., Halfon P., Aslam S., Bremer B., Manns M.P., Cornberg M., Wedemeyer H. (2014). A clinical trial evaluating low HCV RNA viremia in patients treated with triple therapy regimens: implications for patient management using different assays in clinical practice. *Aliment Pharmacol Ther.* **39**, 85-92.

Pawlotsky J.M. (2014). New hepatitis C therapies: the toolbox, strategies, and challenges. *Gastroenterology.* **146**, 1176-1192.

Peiffer K.H., Sarrazin C. (2013). The importance of HCV RNA measurement for tailoring treatment duration. *Dig Liver Dis.* **45** (Suppl. 5), S323-331.

Poordad F., McCone Jr J., Bacon B.R., Bruno S., Manns M.P., Sulkowski M.S., Jacobson I.M., Reddy K.R., Goodman Z.D., Boparai N., DiNubile M.J., Sniukiene V., Brass C.A., Albrecht J.K., Bronowicki J.P., SPRINT-2 Investigators. (2011). Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med.* **364**, 1195-1206.

Pyne M.T., Hillyard D.R. (2013). Evaluation of the Roche COBAS AmpliPrep/COBAS TaqMan HCV Test. *Diagn Microbiol Infect Dis.* **77**, 25-30.

Seeff L.B. (2002). Natural history of chronic hepatitis C. *Hepatology.* **36** (5 Suppl. 1), S35-46.

Vermehren J., Aghemo A., Falconer K., Susser S., Lunghi G., Zeuzem S., Colombo M., Weiland O., Sarrazin C. (2014). Clinical significance of residual viremia detected by two real-time PCR assays for response-guided therapy of HCV genotype 1 infection. *J Hepatol.* **60**, 913-919.

Wiegand J., Neumann K., Böhm S., Weich V., Teuber G., Klinker H., Möller B., Rasenack J., Hinrichsen H., Gerlach T., Spengler U., Buggisch P., Sarrazin C., Berg T. (2011). Importance of minimal residual viremia for relapse prediction in patients with chronic hepatitis C genotype 1 infection. *Clin Infect Dis.* **53**, 1111-1114.

Wiesmann F., Naeth G., Sarrazin C., Berger A., Kaiser R., Ehret R., Knechten H., Braun P. (2014). Variation analysis of six HCV viral load assays using low viremic HCV samples in the range of the clinical decision points for HCV protease inhibitors. *Med Microbiol Immunol.* **204**, 515-525.