Comparison of Performance Characteristics of DxN VERIS System versus Qiagen PCR for HBV Genotype D and HCV Genotype 1b Quantification

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A b s t r a c t

The Beckman Coulter DxN VERIS system is a fully automated, closed molecular diagnostic instrument for viral load quantification of hepatitis B virus and hepatitis C virus. In this study, the analytical performance of this new system was compared to routine diagnostic Qiagen PCR kit by using the same clinical samples. The DxN VERIS system demonstrated a high analytical performance. The DxN VERIS allows random access, which means that samples can be uploaded straight on to the system at any time; so, it provides an improvement of workflow, staff productivity and allows faster turn-around of viral load results.

K e y   w o r d s: Hepatitis B virus, hepatitis C virus, real-time PCR, regression analysis, diagnosis

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are hepatotropic, non-cytopathic viruses able to establish a persistent infection that causes different degrees of hepatic inflammation (chronic hepatitis), leading to the development of liver cirrhosis and hepatocellular carcinoma (HCC) (Caccamo et al. 2014; Easterbrook et al. 2017). Today, the main goal of HBV therapy is to prevent the progression of the disease, improving survival and the quality of life; whereas, for the HCV therapy, the aim is to cure the infection to achieve a sustained virological response and consequently, to prevent HCC development (Akhan et al. 2015). Additionally, viral load (VL) monitoring is important in assessing the therapeutic response, monitoring treatment success and detecting drug-resistant viruses (Singh et al. 2017). There are several commercially available HBV-HCV monitoring assays which are mainly based on real-time polymerase chain reaction (RT-PCR); but, using the most effective, the cheapest and the fastest detecting system plays an important role in diagnosing viral infections. Although these techniques are preferred due to their excellent analytical sensitivity, specificity, accuracy, and broad dynamic range of linear quantification, they require many steps and consumables in the qualified laboratories (Portilho et al. 2015; Wu et al. 2017). VL assays also need to be batched as they arrive in the laboratory. Even a small change in the efficiency of the amplification can lead to striking differences in the amount final product. These reasons indicate the necessity of inventing fully standardized, reproducible and sensitive new assays for monitoring of HBV and HCV infections.

The aim of the study was to compare the analytical performance of random access testing (Beckman Coulter DxN VERIS) with routine diagnostic PCR kit (artus Qiagen).

A total of 67 chronic hepatitis B (CHB) patients with genotype D and 44 chronic hepatitis C (CHC) patients infected with genotype 1b from Kocaeli province in Marmara region of Turkey were analyzed in parallel with the use of DxN VERIS and Qiagen systems

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Table I
The Qiagen HBV DNA and HCV RNA levels in sampling.

Pattern of HBV sampling	HBV DNA (IU/ml), n/total (%)	Pattern of HCV sampling	HCV RNA (IU/ml), n/total (%)
Negative	12/67 (18%)	Negative	11/44 (25%)
$< 10^1$	14/67 (21%)	$> 10^1$	1/44 (2%)
$> 10^2$	9/67 (13%)	$> 10^2$	1/44 (2%)
$> 10^3$	13/67 (20%)	$> 10^4$	2/44 (5%)
$> 10^4$	4/67 (6%)	$>10^5$	6/44 (14%)
$> 10^5$	7/67 (10%)	$> 10^6$	14/44 (32%)
$> 10^6$	4/67 (6%)	$>10^7$	9/44 (20%)
$> 10^8$	4/67 (6%)	–	–

synchronously. Qiagen HBV DNA and HCV RNA levels in the samples were in the range from negative to $> 10^8$ IU/ml and $> 10^7$ IU/ml, respectively, and they are displayed in Table I. The PCR-negative samples were previously diagnosed as taken from the HBV and HCV carriers and their negativities were provided by antiviral treatment. The most predominant genotypes in Turkey are HBV genotype D and HCV genotype 1b; therefore, we have only involved these genotypes in our study (Karatas et al. 2018). The selected blood samples were immediately centrifuged, aliquoted, and then, stored at –80°C until use. The diagnosis of chronic hepatitis B and C was made on the basis of biochemical, serological, virological and histological data according to the EASL guidelines (EASL 2017a, 2017b). The samples were analyzed by using both fully automated DxN VERIS kits (Beckman Coulter DxN VERIS HBV/HCV kits, Nyon, Switzerland) and Qiagen kits (artus HBV/HCV QS RGQ kit, Qiagen, Hilden, Germany) on the same day according to the manufacturer's recommendations. DxN VERIS HBV/HCV assays have quantification ranges from $10–10^9$ IU/ml and $12–10^8$ IU/ml, respectively as it is stated in the package inserts. The relationship between VL quantifications measured by both DxN VERIS and Qiagen assays were analyzed by Passing-Bablok (PB) regression method. Bland Altman plot (BAP) design was used to calculate statistical limits, correlation and standard deviation (SD) of the differences between two types of measurement. To control the distribution of the differences and other properties between quantitative values, a graphical approach was used. Statistical analysis of the study was made with the Analyse-it Software program (Analyse-it Ltd. v 4.60, Microsoft Corp. Leeds, UK).

According to HBV BAP (DxN VERIS combined Qiagen HBV log IU/ml), the specificity resulted in 95% LoA (limits of agreement) [CL (confidence level) 95% = –1.59–0.61], the correlation was equal to 0.97 with SD 0.55 and a mean SD = –0.47. HBV PB indicated DxN VERIS combined = –1.179 + 1.153 Qiagen

log IU/ml with the correlation equal to 0.97. HBV plots for PB and BAP are shown in Fig. 1.

For HCV analysis, BAP (DxN VERIS combined Qiagen HCV log IU/ml) illustrated the specificity of 95% LoA (CL 95% = –1.59–0.72), the correlation of 0.90 with SD 0.59 and a mean SD = –0.43. HCV PB analysis indicated DxN VERIS combined = –0.3394 + 0.8602 Qiagen log IU/ml with the correlation of 0.90. HCV plots for PB and BAP are shown in Fig. 2.

Viral nucleic acid detection is the gold standard for the detection of viral genomes in clinical samples. COBAS Ampliprep, artus Qiagen and Abbott real-time PCR assays are currently the most frequently used platforms worldwide in this field. DxN VERIS systems were mainly compared to COBAS and Abbott systems rather than to Qiagen kits (Saune et al. 2016; Patrick et al. 2017; Park et al. 2019). Studies comparing DxN VERIS HBV to Roche HBV and to Abbott real-time HBV illustrate the average bias that was determined as being equal to –0.26 (95% Cl: –0.37 to –0.15) and –0.36 (95% Cl: –0.43 to –0.29), respectively in BAP. This was equal to –0.32 log IU/ml in another study (Robert et al. 2014), and respectively to –0.47 log IU/ml in our study (Williams et al. 2014; Park et al. 2019). In the present study, we compared DxN VERIS with Qiagen HBV and HCV and their clinical performances displayed a strong correlation (95%) similar to one another study (Micheli et al. 2016). Currently, the main therapy is based on Peg-IFN and nucleos(t)ide analogues for CHB. However, the main clinical challenge is the development of antiviral resistance, since long-term therapeutic regimens are given to the majority of these patients (EASL 2017b). While monitoring VL, HBV breakthrough that is demonstrated by a sudden increase in DNA level (from $> 1 \log_{10}$ IU/ml to $> 1 \times 10^5$ IU/ml) can be observed (Braun et al. 2017). Early detection of such viral breakthrough is important because the analysis of the HBV gene mutations responsible for drug resistance is a part of managing clinical treatment. The random access testing can provide effective surveillance
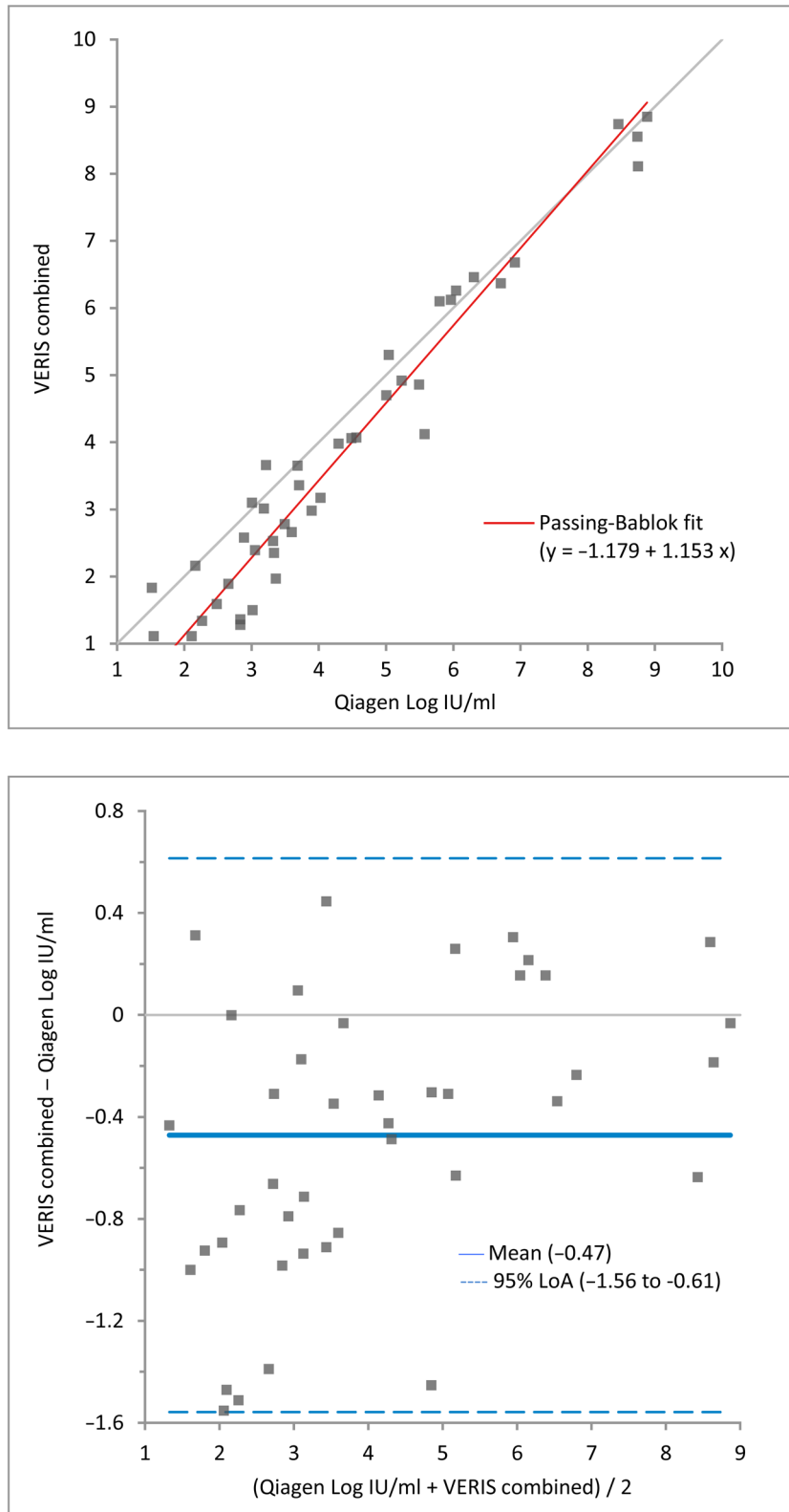
Fig. 1. HBV plots for Passing-Bablok (upper) and Bland Altman analysis (lower).

for CHB treatment. Similarly, monitoring of HCV RNA during treatment is required to assess the success of the treatment and to detect any breakthrough related to viral resistance. As the main goal of therapy for HCV is to cure the infection in order to prevent the development of HCC and complications related with HCV related liver diseases, random access system enables faster turn-around of VL results to physicians and allows early detection of possible resistance. Therefore, DxN VERIS system may be a new solution as it enables
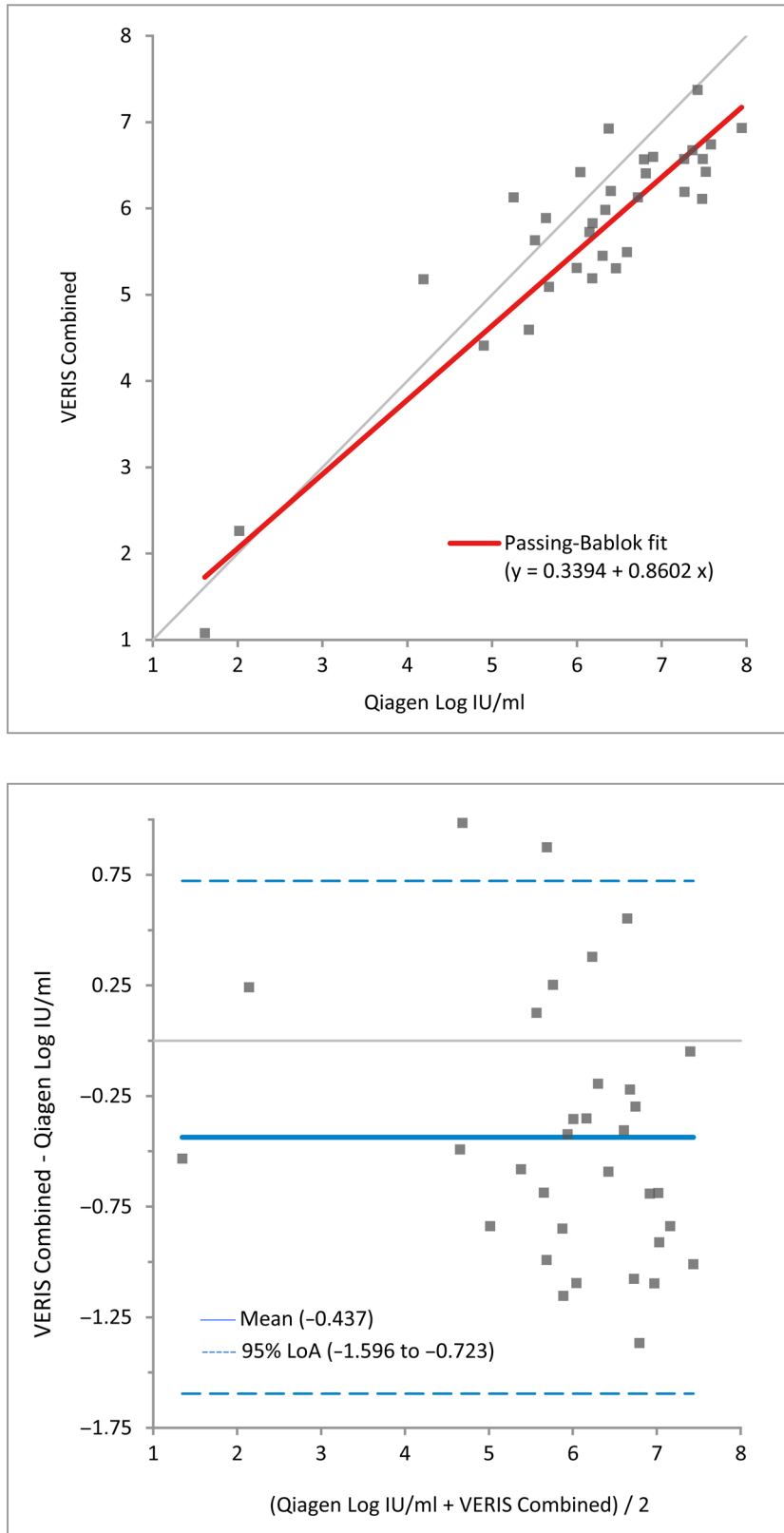
Fig. 2. HCV plots for Passing-Bablok (upper) and Bland Altman analysis (lower).

same-day turn-around results to help the health system improving patient management. None of the other platforms are true single-sample to answer and random-access testing. This is the most important advantage of DxN VERIS system for us because medical reports can be transformed within a short time to different departments and physicians that could better manage the diseases with antiviral therapy in a short time.

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