Improved management of hepatitis D: Standardized quantification of HDV RNA

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» INTRODUCTION

HDV is a small, defective RNA virus that can infect only individuals who have HBV; worldwide more than 15 million people are co-infected. HDV increases the severity of chronic HBV infection, frequently leading to cirrhosis, hepatic decompensation or hepatocellular carcinoma. The prevalence of HDV is declining in some endemic areas but increasing in northern and central Europe because of immigration. Interferon-alpha is currently the only available treatment option leading to suppression of HDV RNA in 25 % - 30 % of patients [1]. However, pegylated interferon therapy is associated with sometimes severe side effects and only a minority of patients is eligible for treatment. Novel alternative treatment options are currently in early clinical trials. Confirmation of HDV infection, as well as standardized monitoring of viral load, is urgently needed in order to personalize patient management.

» OBJECTIVES

The majority of nucleic assay amplification tests for viral load monitoring of HDV RNA are developed in-house based on real-time PCR using internal standards of different origin resulting in different measuring units. This issue impedes comparability and was greatly improved with the establishment of the 1st WHO standard for HDV RNA [2]. The novel RoboGene® HDV RNA Quantification Kit 2.0 is worldwide the first CE-IVD-certified One-Step Real-Time PCR kit for quantification of HDV RNA applying the 1st WHO standard for HDV RNA. Thus, analysis of patient samples using the kit enables expression of clinical relevant data in IU/ml. This is the basis for investigation of treatment efficacy, patients would benefit from. To evaluate the performance of the novel real-time PCR kit, the RoboGene® HDV RNA Quantification Kit 2.0 has been compared to an earlier published in-house method used for routine diagnostics.

» MATERIAL AND METHODS

Using the utility channel of the Cobas Ampliprep/TaqMan system platform, the earlier published in-house assay uses a protocol for TaqMan-based HDV RNA quantification after automatic extraction of RNA by the Ampliprep system [3]. The method uses a plasmid-based standard of HDV cDNA cloned in pUC19 and has a specified limit of detection of 15 copies/ml [4]. The RoboGene® HDV RNA Quantification Kit 2.0 is valid with the INSTANT Virus RNA/DNA Kit for viral RNA extraction. The kit provides ready-to-use standard strips that are stably coated with defined amounts of HDV standard RNA. They have been calibrated using the 1st WHO standard for HDV RNA. Real-Time PCR runs were performed on the LightCycler® 480 II. In order to achieve comparability of the two methods different dilutions of the 1st WHO standard for HDV RNA were analyzed. Furthermore, samples of 15 HDV IgG positive (genotype 1) and 5 HDV IgG negative patients were analyzed at the Medical School of Hannover using both workflows. The study included a longitudinal perspective as samples were collected before as well as 12 and 48 weeks following treatment with pegylated interferon alpha. To analyze the diagnostic sensitivity and linearity data of the patient samples was correlated.

» RESULTS

Data represented in Table 1 include a dilution series that have been created during evaluation of the RoboGene® HDV RNA Quantification Kit 2.0 according to the CTS for in vitro diagnostic medical devices (2009/886/EC). The limit of detection has been determined as 10 IU/ml. Following analyses of the 1st WHO standard the limit of detection of the in-house assay was determined as approximately 500 IU/ml (Table 1).

Concentration [IU/ml]	Detection rate	
	RoboGene®	In-house assay
100.000	25/25	6/6
10.000	25/25	6/6
1.000	25/25	6/6
500	50/50	4/6
100	50/50	1/6
50	51/51	-
25	48/48	-

Table 1 | Referencing of the two assays using the 1st WHO standard for HDV RNA in order to determine a limit of detection

For diagnostic evaluation results from the kit were consistent with those from the applied in-house method before starting point of therapeutic treatment. Following 12 weeks 80 % of the patient samples were determined as positive by the kit, but 67 % using the in-house method. 48 weeks following starting point of treatment 67 % and 60 % were positively tested using the kit and in-house, respectively (Figure 1).
The linear regression analysis of the obtained data showed a very high degree of correlation (R^2= 0.89) between both assays and is represented in Figure 2.

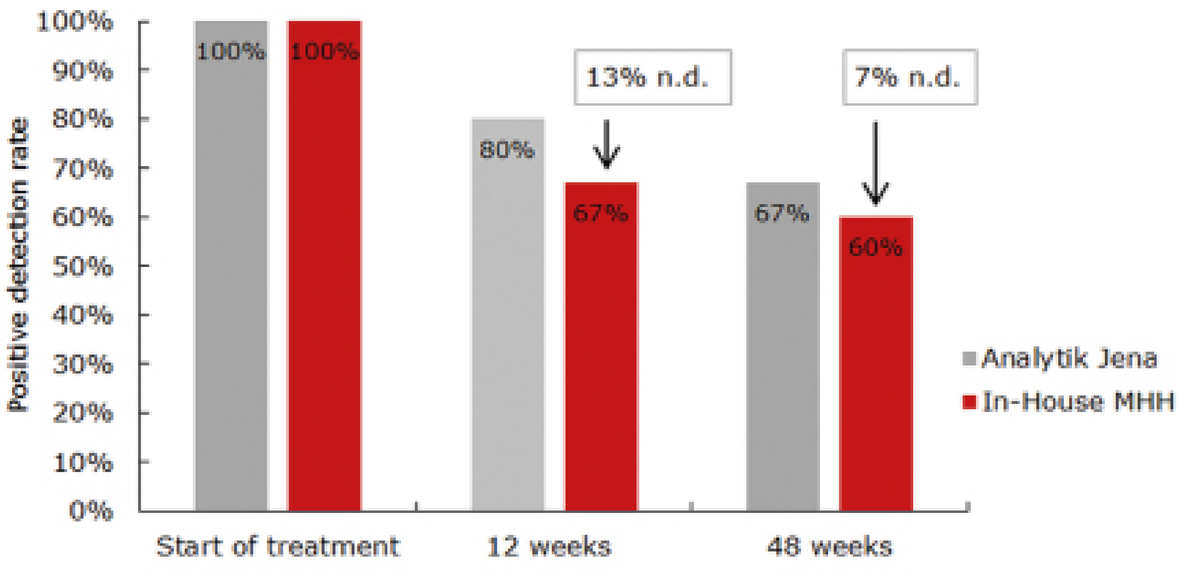

Figure 1 | Positive detection rate of HDV positive patients (n=15) before, 12 and 48 weeks following treatment using Analytik Jena's RoboGene® Kit vs. In-house method of MHH

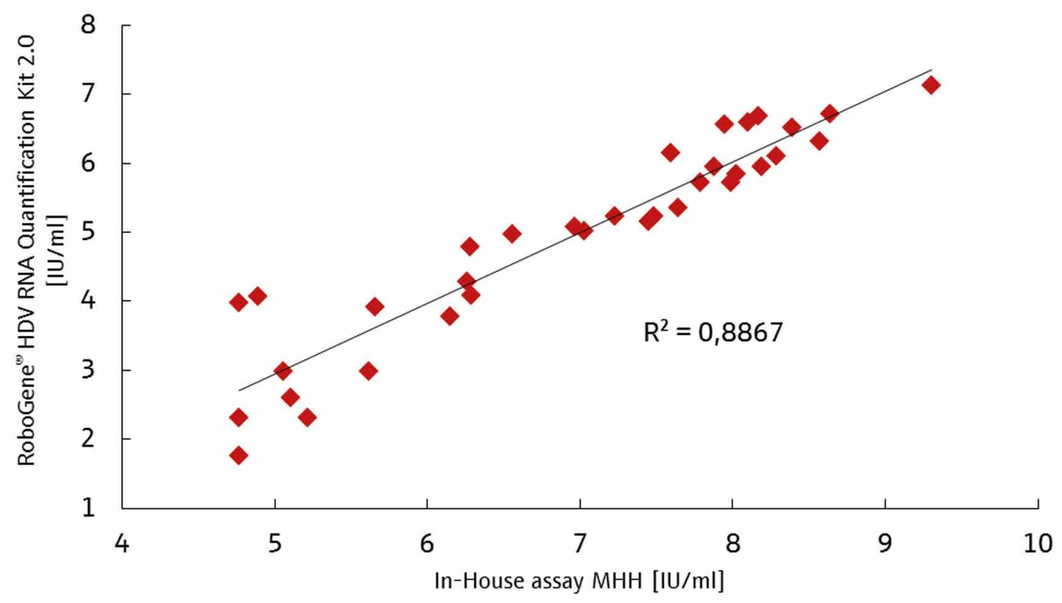

Figure 2 | Diagnostic evaluation: comparison of the RoboGene® HDV RNA Quantification Kit 2.0 to the in-house method

» CONCLUSIONS

Hepatitis D represents a major and life-threatening health burden in certain areas of the world. Confirmation of hepatitis infection, as well as standardized assessment of antiviral treatment effectiveness may improve patient management in the future. It is worth monitoring kinetics of HDV RNA in order to identify patients who are true non-responders to therapy and individuals who would benefit from extended treatment. Therefore, the RoboGene® HDV RNA Quantification Kit 2.0 is an optimal tool as it can be reliably used to confirm HDV infection and to determine treatment efficacy. Beside featuring a strong limit of detection clinical investigators benefit from the provided internal control applicable as extraction and amplification control when monitoring HDV RNA levels. The kit proved to reduce the rate of false-negative results enabling a trustworthy monitoring of HDV RNA level until almost clearance of HDV RNA.

Reference:
1 Wedemeyer H, Manns MP Nat Rev Gastroenterol Hepatol 2010 7:31-40
2 Chudy M, Hanschmann KM, Bozdayi M, Kress J, Nübling CM 2013 WHO/BS/2013.2227
3 Mederacke I, Bremer B, Heidrich B, Kirschner J, Deterding K, Bock T, Wursthorn K, Manns MP, Wedemeyer H J Clin Microbiol 2010 48(6):2022-9
4 Taranta A, Rogalska-Taranta M, Gutierrez R, Manns MP, Bock M, Wursthorn K J Clin virol 2014 61:268-288

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