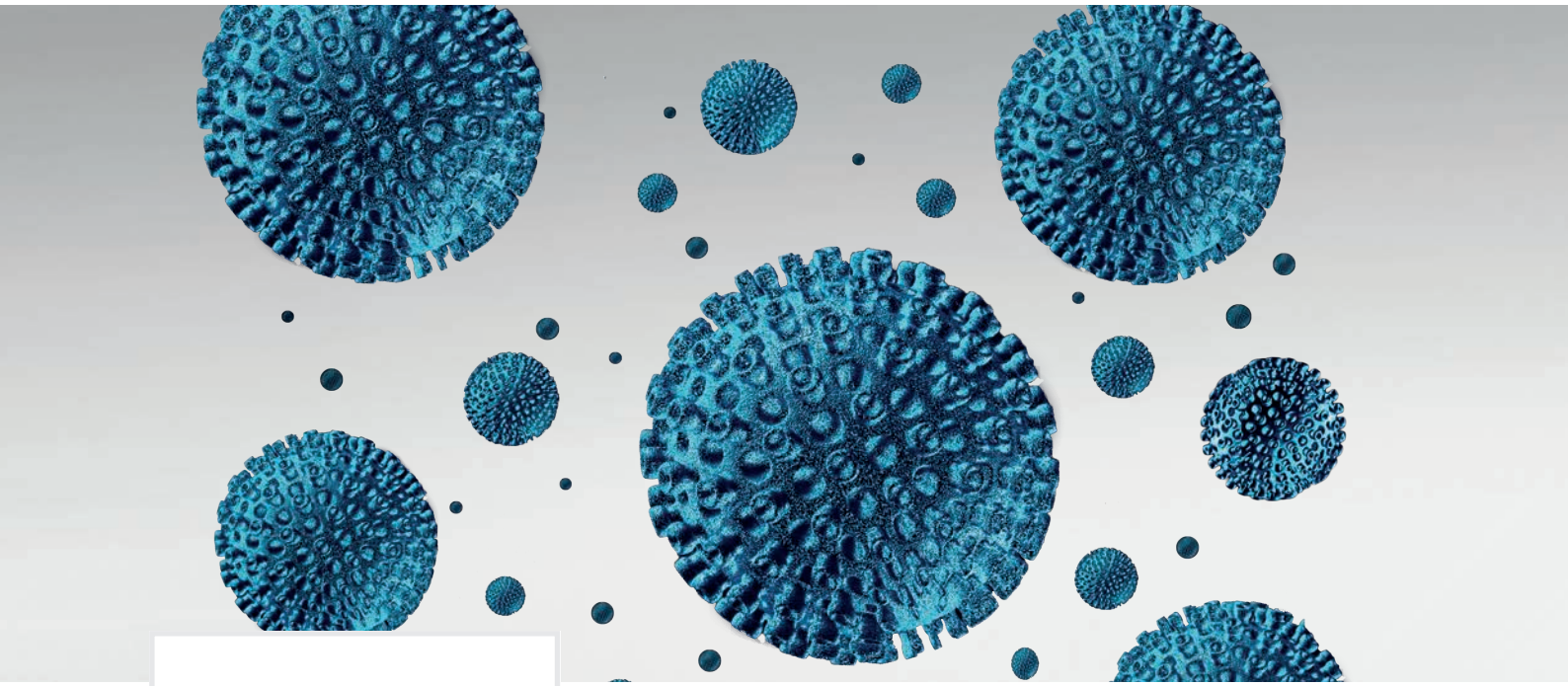
Application Note · RoboGene® HDV RNA Quantification Kit 2.0



Challenge

Reliable detection of all worldwide identified genotypes of the Hepatitis Delta Virus

Solution

RoboGene® HDV RNA Quantification Kit 2.0

Reliable Detection of HDV RNA Independent of the Genotype

Introduction

Hepatitis Delta Virus (HDV) infection is considered to be the most severe hepatic disease; worldwide more than 15 million people are co-infected. Determining HDV RNA is important to distinguish an active from a non-active infection. HDV RNA is the marker of active HDV infection and can guide the clinician on his recommendation of therapy. 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 sometimes associated with severe side effects and only a minority of patients are eligible for treatment. Novel alternative treatment options are currently in early clinical trials.

At least eight Hepatitis Delta Virus (HDV) genotypes have been identified with a specific geographic distribution. HDV genotype 1 is the most frequent and is distributed throughout the entire world. HDV-2 and -4 are found in Far Eastern Asia, HDV-3 in northern South America. However, because of migration from endemic countries, confirmation of HDV infection, as well as standardized monitoring of viral load independent of the present genotype, is urgently needed in order to personalize patient management.

To further evaluate the performance of the RoboGene® HDV RNA Quantification Kit 2.0, which is referenced to the 1st WHO standard for HDV RNA based on Genotype 1, an additional study was performed to prove the genetic variability of HDV detected by the kit.

analytikjena
An Endress+Hauser Company

Materials and Methods

HDV positive samples of all different genotypes were selected. Five concentrations from consecutive 1:10 serial dilutions were prepared (neat, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}). HDV RNA eluates were quantified with the FNRL-HDV technique [2] and the RoboGene® Kit on ABI 7500 Fast at the French National Reference Laboratory for HDV, France. One sample, identified as HDV-3 by a qualitative in-house method of FMT-HVD Laboratory, Brazil [3], was diluted at a ratio of 1:5 and used for an analysis with RoboGene® Kit.

Results and Discussion

According to this particular panel of genotype samples, the RoboGene® Kit showed excellent repeatability and sensitivity. The kit was able to detect all strains of the panel, showing good specificity for the different genotypes (Fig. 1 - 8). Due to the high genetic variability of HDV genus, fluctuations in quantification of non-HDV-1 samples are very common.
Comparability is impeded due to the lack of WHO standards for HDV RNA covering different genotypes. However, very good linearity from the five dilution points of each sample was observed, independent of the genotype with a coefficient of correlation R^2 ranging from 0.9645 to 0.9994. The excellent sensitivity of the RoboGene® Kit (LOD 8 IU/ml for use of ABI 7500 Fast) was proven during this evaluation, as the kit was able to systematically detect and quantify very low HDV viral loads.

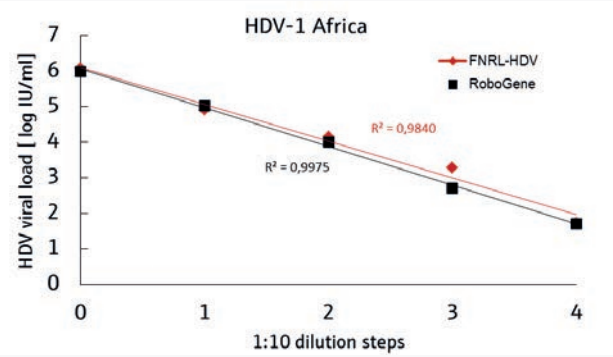


Fig. 1: HDV-1 Africa (starting concentration 1.67 x 10^6 IU/ml)

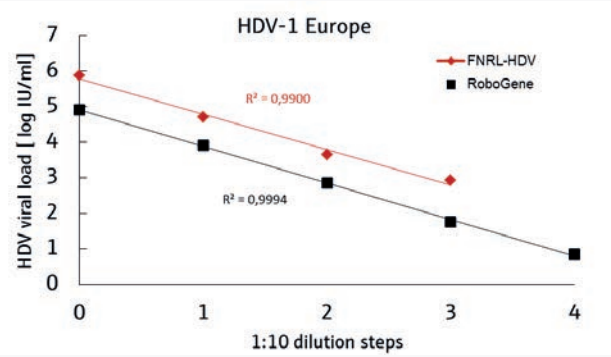


Fig. 2: HDV-1 Europe (starting concentration 9.22 x 10^5 IU/ml)

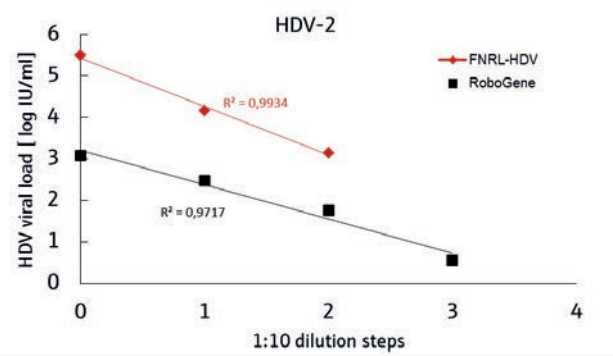


Fig. 3: HDV-2 (starting concentration 2.50 x 10^5 IU/ml)

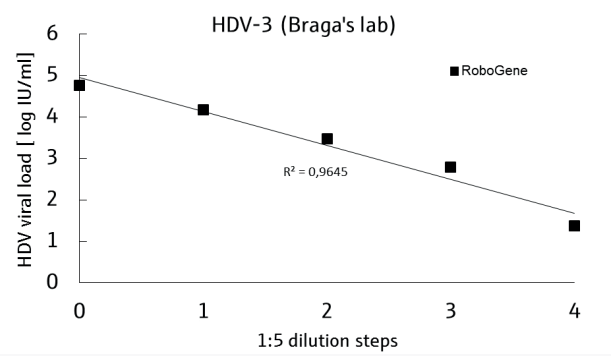


Fig. 4: HDV-3 (starting concentration 6.00 x 10^4 IU/ml)

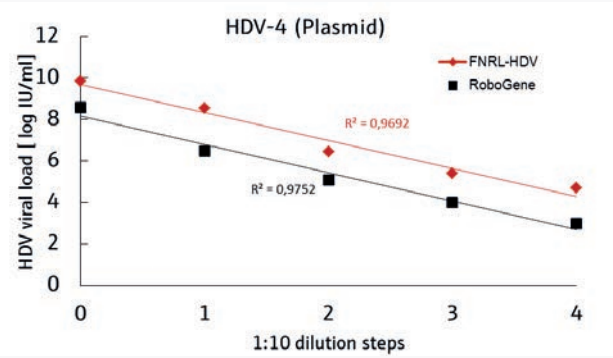


Fig. 5: HDV-4 plasmid (starting concentration 1 x 10^{10} IU/ml)

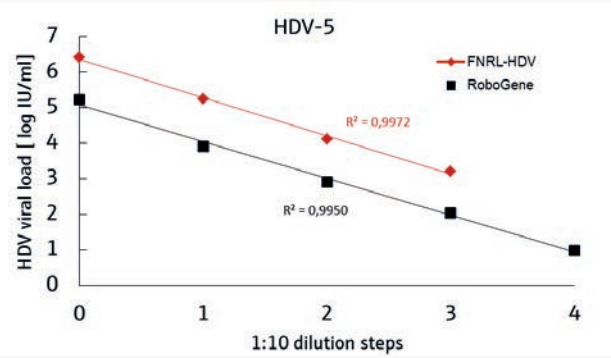


Fig. 6: HDV-5 (starting concentration 1.38 x 10^6 IU/ml)

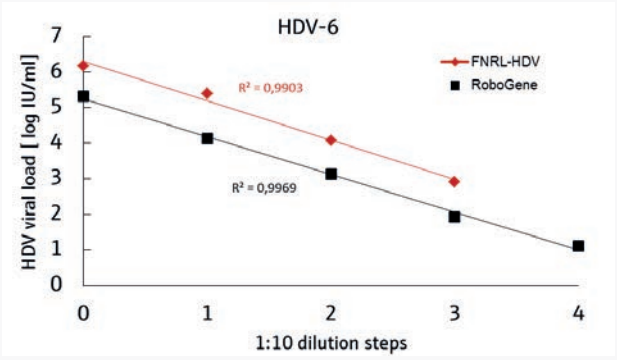


Fig. 7: HDV-6 (starting concentration 1.78 x 10^6 IU/ml)

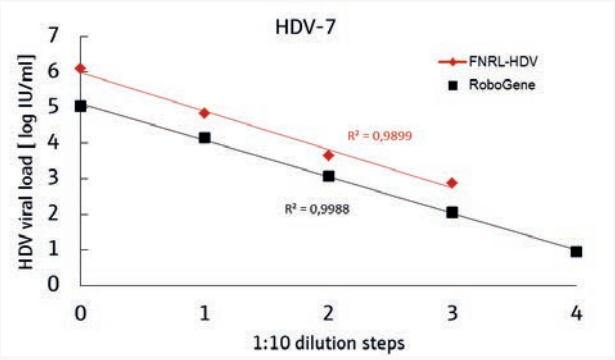


Fig. 8: HDV-7 (starting concentration 3.93 x 10^6 IU/ml)

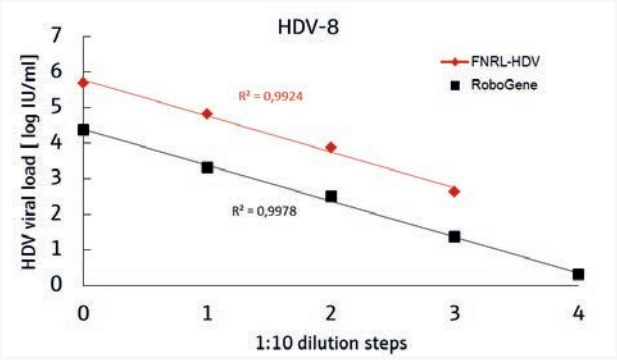


Fig. 9: HDV-8 (starting concentration 8.10 x 10^5 IU/ml)

Conclusion

Hepatitis D represents a major and life-threatening health burden in certain areas of the world. The study has proven that the kit is a reliable tool in investigating and quantifying patient samples offering excellent sensitivity, independent of the occurring HDV genotype.

References

[1] Wedemeyer H, Manns MP Nat Rev Gastroenterol Hepatol (2010) 7:31-40
[2] Le Gal F et al. J Clin Microbiol (2005) 43 (5):2363-9
[3] Kay A et al. J Viral Hepat (2014) 21(12):921-4

Acknowledgements

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Headquarters
Analytik Jena AG
Konrad-Zuse-Strasse 1
07745 Jena · Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279

info@analytik-jena.com
www.analytik-jena.com

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