Diplomarbeit

Comparison of automated extraction platforms on quantification of plasma hepatitis D virus RNA

eingereicht von

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Graz, 19. März 2020

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Abbreviations

ALT	Alanine amino transferase
CF	Correction factor
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B virus
HDV	Hepatitis D virus
IU	International units
LOD	Limit of detection
PCR	Polymerase chain reaction
qPCR	Real time quantitative polymerase chain reaction
RNA	Ribonucleic acid
WHO	World Health Organization

Zusammenfassung

Hintergrund: Das Hepatitis D Virus (HDV) verursacht die schwerste Form der viralen Hepatitiden und benötigt daher eine zuverlässige Diagnostik. Das Ziel dieser Studie war es, Ergebnisse der quantitativen Bestimmung der HDV RNA Konzentration im Plasma vergleichbar zu machen, die mit verschiedenen Extraktionsinstrumenten und dem CE/IVD-zertifizierten RoboGene® HDV RNA Quantification Kit 2.0 (Analytik Jena AG) erhalten wurden.

Materialien und Methoden: Alle Proben wurden entweder mit dem eMAG® (bioMérieux S.A., Marcy l´Etoile, Frankreich) oder dem MagNA Pure 24 (Roche Molecular Diagnostics, Rotkreuz, Schweiz) extrahiert. Amplifikation und Detektion erfolgten mit dem LightCycler 480II Instrument (Roche). Nach Berechnung der Korrekturfaktoren mit dem 1. WHO internationalen Standard für HDV RNA wurden die Nachweisgrenzen für beide Testsysteme bestimmt. Danach wurde die Testgenauigkeit mit Referenzmaterial überprüft und die klinische Leistung mit 30 Plasmaproben (5 davon HDV RNA negativ und 25 positiv) getestet.

Ergebnisse: Der Korrekturfaktor wurde mit 922 für das Testsystem mit der eMAG® Plattform und 190 für das Testsystem mit der MagNA Pure 24 Plattform berechnet. Die Nachweisgrenzen wurden mit 2,65 $\log_{10}$ IU/mL bzw. 2,37 $\log_{10}$ IU/mL bestimmt. Beide Testsysteme zeigten korrekte Ergebnisse bei der Überprüfung mit dem Referenzmaterial. Die Testung der klinischen Leistung ergab bezüglich der HDV RNA-negativen Plasmaproben ein korrektes Resultat mit beiden Testsystemen. Von den 25 HDV RNA-positiven klinischen Proben wurden 15 mit beiden Testsystemen positiv getestet, 2 ergaben ein diskrepantes Ergebnis und 8 konnten mit beiden Testsystemen nicht detektiert werden.

Schlussfolgerungen: Die Quantifizierung der HDV-RNA im Plasma hängt von der verwendeten Extraktionsplattform ab. Um Ergebnisse vergleichbar zu machen, muss für jedes Testsystem der entsprechende Korrekturfaktor bestimmt werden. Die Harmonisierung der Ergebnisse ist essenziell, um die anti-HDV-Therapie zu überwachen und Ergebnisse aus klinischen Studien zu vergleichen.

Abstract

Background: Hepatitis D (HDV) is the most severe and rapidly progressive form of chronic viral hepatitis needing a reliable diagnostic approach. The aim of this study was to make results comparable when using the CE/IVD-labeled RoboGene® HDV RNA Quantification Kit 2.0 (Analytik Jena AG) with two different laboratory settings.

Materials and Methods: All samples were extracted either with the eMAG® (bioMérieux S.A., Marcy l´Etoile, France) or the MagNA Pure 24 (Roche Molecular Diagnostics, Rotkreuz, Switzerland) platform. Amplification and detection were performed on the LightCycler 480II instrument (Roche). After determination of correction factors (CF) utilizing the 1st WHO International Standard for HDV RNA, the limit of detection (LOD) was determined for both test systems followed by accuracy testing using a reference panel. Furthermore, 30 clinical plasma samples (25 of them HDV RNA positive and 5 of them HDV RNA negative) were tested and results compared.

Results: The CF was 922 for the test system including the eMAG® and 190 for the test system including the MagNA Pure 24. LODs were found to be 2.65 $\log_{10}$ IU/mL and 2.37 $\log_{10}$ IU/mL, respectively. One member of the reference panel containing 2.03 $\log_{10}$ IU/mL tested negative with both test systems and another member containing 3.11 $\log_{10}$ IU/mL gave a negative result with the test system including the eMAG®. Of 25 HDV RNA positive samples, 15 tested positive with both test systems, 2 gave discrepant results, and in 8 samples, HDV RNA was not detected with both of the methods. All clinical samples that did not contain HDV RNA tested negative with both test systems.

Conclusions: Quantification of plasma HDV RNA depends on the extraction platform used. To make results comparable, it is mandatory to determine the specific CF for the test system employed. Harmonization of results is essential for monitoring patients undergoing anti-HDV therapy and making results obtained from clinical studies comparable.

1 Introduction

Hepatitis D virus (HDV) is a small RNA virus, which belongs to the family *Deltaviridae*, and it is one of the five triggers of viral hepatitis (1,2). It was discovered by Rizzetto and colleagues in the mid-1970s in patients with HBV and severe liver damage (3). Figure 1 shows the HDV virion with its hepatitis B virus (HBV) helper virus. HDV is an incomplete satellite virus with an envelope consisting of HBV surface antigen (HBsAg) which is the reason for HDV needing a host cell primarily infected with HBV to develop, no matter being a superinfection or co-infection (1,4). Speaking of co-infections, a combination of HBV-HDV is supposed to be the most severe form of viral hepatitis to treat and also it is more likely to develop hepatocellular carcinoma (HCC) and cirrhosis by being infected with both viruses than with HBV alone (3).

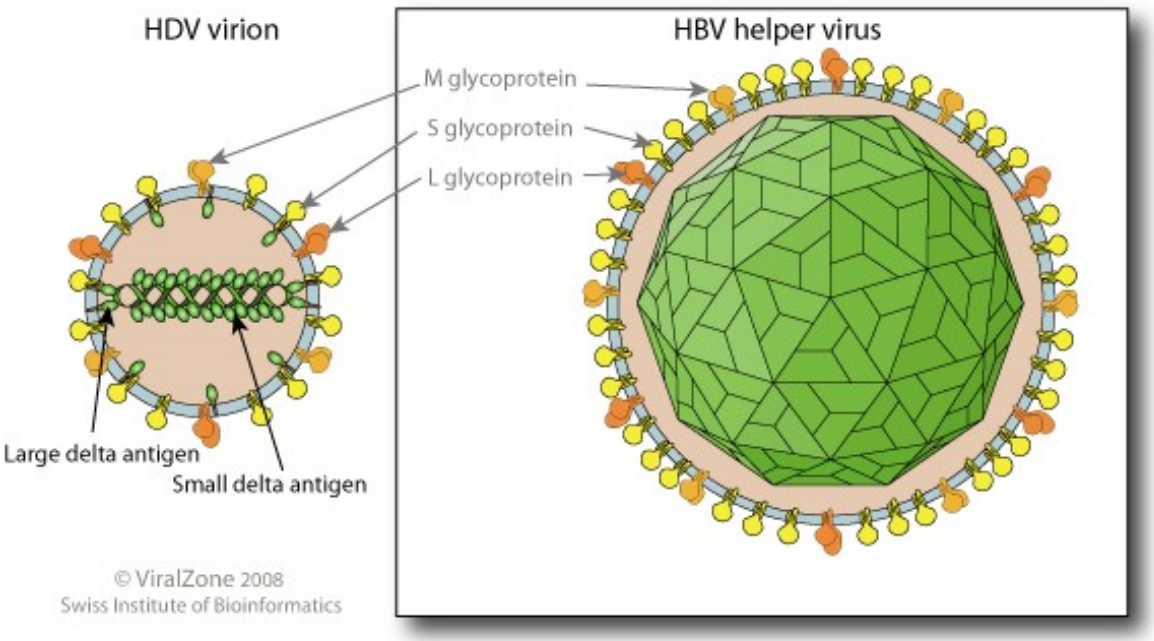

Fig. 1. The HDV virion with its HBV helper virus (from: https://viralzone.expasy.org/175) (accessed 23.11.2018).

1.1 Prevalence and transmission of HDV

Worldwide, there are 350 million people chronically infected with HBV and approximately 15 to 20 million are co- or superinfected with HDV (1,3). Because of effective vaccination programs in developed countries, HDV infections decreased. However, in some western countries such as Italy, Germany, the United Kingdom, Turkey (4), France, and Spain (1) the number of HDV infections is currently increasing, probably due to immigration from persistent HDV-reservoirs (4) such as Africa and eastern Europe (1). Mongolia and Pakistan are currently considered as countries with the highest HDV prevalence worldwide (Fig. 2)

HDV transmission routes are identical to those of HBV. The major transmission routes are parenteral, mainly through blood and body fluids, sexual, and vertical. Globally, most hepatitis B infections occur through mother-to-child transmission. Among adults, transmission happens mainly through sexual intercourse. In Austria, a high number of HBV infections occur through unsafe injection practices.

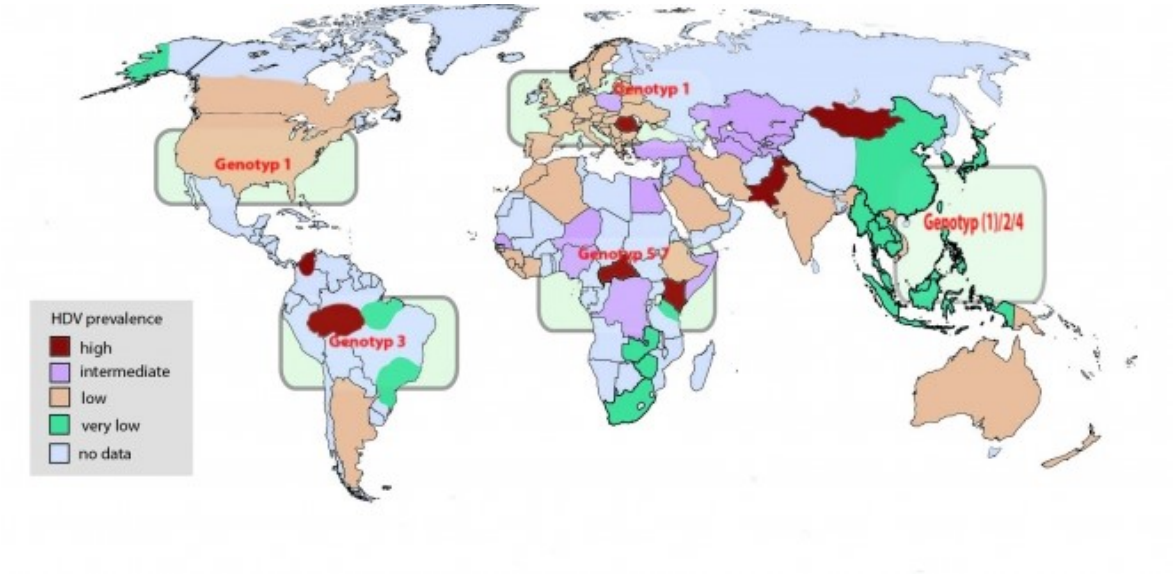

Fig. 2. The global distribution of HDV (figure kindly provided by Heiner Wedemeyer, Hepatitis D International Network).

1.2 The HDV genome and viral structure

HDV is a small RNA virus (Fig. 3). With approximately 1682 base pairs, it contains the smallest genome among all known RNA viruses. It consists of a single-stranded circular RNA (ssRNA). HDV is shaped by a spherical lipoprotein envelope which contains HBsAg (2). It can be distinguished between eight different genotypes of HDV (HDV-1 through HDV-8) (2), but the most common worldwide is genotype 1, which can be found especially in Europe and North America (5).

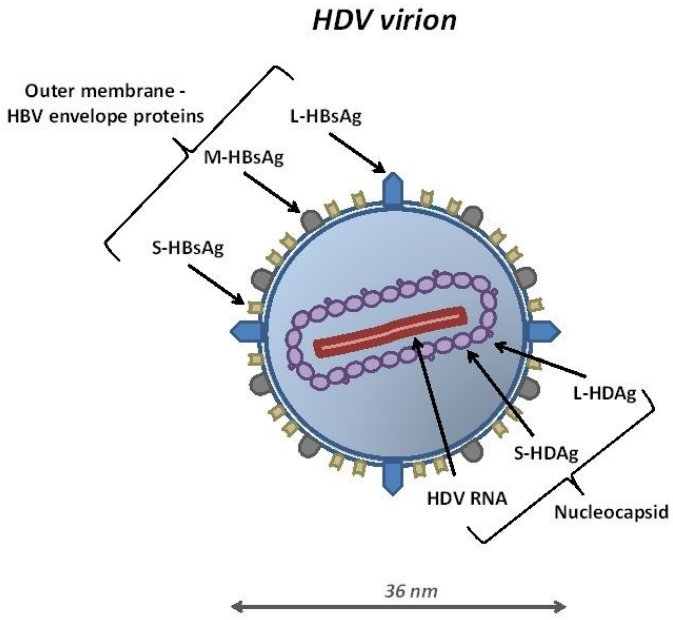

Fig. 3. The HDV virion (from: https://www.intechopen.com/books/drug-discovery-and-development-from-molecules-to-medicine/current-management-and-novel-therapeutic-strategies-to-combat-chronic-delta-hepatitis) (accessed 28.12.2018).

1.3 Diagnostics

In a first step, HBsAg positive patients have to be examined for anti-HDV antibodies (IgG). In a second step, those patients must be screened for HDV RNA in EDTA plasma. This must be done to distinguish between antibodies as a marker for an active infection (HDV RNA positive) or antibodies as a serological scar (HDV RNA negative). This is important for HBsAg-positive patients with liver damage and elevated alanine amino transferase (ALT) (2). The diagnostic algorithm is shown in Figure 4.

Detection of HDV RNA in EDTA plasma is usually done by real-time polymerase chain reaction (qPCR) which allows for determination of HDV viral load.

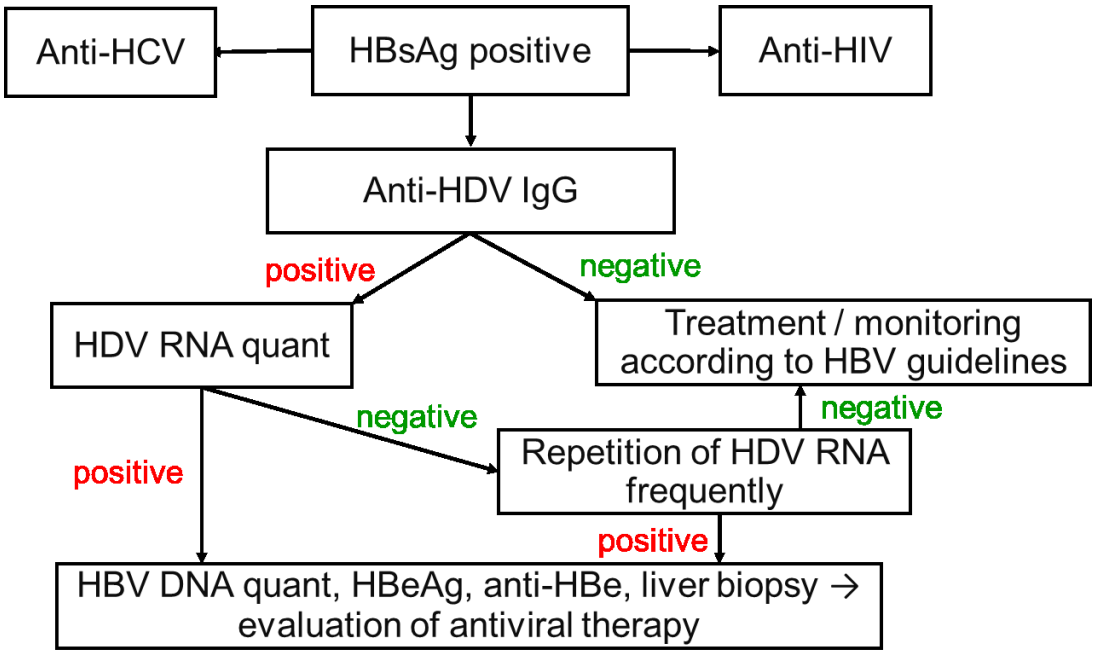

Fig. 4. Diagnostic algorithm (figure kindly provided by Heiner Wedemeyer, Hepatitis D International Network).

1.4 Therapy of HDV

Today, pegylated interferon alfa (pegIFN-α) is the only drug which is approved for the treatment of chronic hepatitis D. Success of therapy is characterized with undetectable HDV RNA in EDTA plasma. Therapy endpoints are suppression of HDV replication, ALT normalization, and decreased liver inflammation. It is intended to achieve a total loss of HBsAg plus a persistent suppression of HDV RNA, which is called complete virological response, but it has been shown that the success rate is very low (6). More recent therapies under evaluation include entry inhibitors (e.g. Myrcludex-B), inhibitors of HBsAg release (nucleic acid polymers), and prenylation inhibitors of the large HDV antigen (7–9).

2 Objectives

Reliable detection and quantification of HDV RNA are essential for diagnosis of hepatitis D and monitoring of anti-HDV therapy. Quantification of HDV RNA may vary depending on the test system used.

In this study, results obtained with the IVD/CE-labeled RoboGene® HDV RNA Quantification Kit 2.0 (Analytik Jena AG, Jena, Germany) in combination with two different automated nucleic acid extraction platforms were compared. Reference material and clinical plasma samples were used.

3 Materials and Methods

3.1 Analytical performance

3.1.1 Determination of the correction factor

In a first step, the correction factor for each combination was determined with a 0.5 $\log_{10}$ dilution series utilizing the 1st WHO International Standard for HDV RNA containing a nominal concentration of 5.76 $\log_{10}$ starting at 5.26 $\log_{10}$ IU/mL (0.5 $\log_{10}$ under the initial concentration). Reference material dilutions were analyzed with both assays. Each dilution was analyzed in triplicate. The correction factor was calculated by correlating the HDV RNA reference material concentration (in IU/mL of the 1st WHO International Standard for HDV RNA) expected against the concentration (in copies/reaction of each combination) obtained (Figure 5). Correction factors were applied for all quantitative results in this study.

$$\text{Correction Factor} = \frac{\text{given concentration [IU/mL]}}{\text{estimated concentration [copies/reaction]}}$$

Fig. 5. Formula used for calculation of the correction factor.

3.1.2 Determination of the limit of detection

The limit of detection (LOD) was determined by analyzing a dilution series of the 1st WHO International Standard for HDV RNA starting with a dilution containing an HDV RNA concentration of 2.76 $\log_{10}$ IU/mL followed by 8 additional 2-fold dilutions and 1 negative control (NC; HDV negative plasma). The limit of detection was calculated by probit analysis.

3.1.3 Accuracy testing with the QCMD 2018 Hepatitis D Virus Program

For accuracy, members of the QCMD (Quality Control for Molecular Diagnostics) 2018 Hepatitis D Virus EQA Program were tested. The panel composition and the results expected are shown in Table 1.

Table 1
QCMD 2018 Hepatitis D Virus panel composition and results expected.

Sample Code	Sample Content	Matrix	Consensus[a] (IU/mL) Log_{10}
HDV 18S-01	HDV (Clinical Sample)	Plasma	2.03
HDV 18S-02	HDV (Clinical Sample: N6358)	Plasma	5.01
HDV 18S-03	HDV (Clinical Sample: N6358)	Plasma	3.05
HDV 18S-04	HDV (International Standard)	Plasma	4.10
HDV 18S-05	HDV (International Standard)	Plasma	4.11
HDV 18S-06	HDV (Clinical Sample)	Plasma	3.11
HDV 18S-07	HDV (Clinical Sample: N6358)	Plasma	4.12
HDV 18S-08	HDV (Clinical Sample)	Plasma	4.06
HDV 18S-09	HDV Negative	Plasma	TND[b]

[a]Mean consensus (log_{10} IU/mL) calculated from data returned by participants with outliers removed

[b]TND, target not detected

3.2 Clinical performance

Anonymized left-over samples obtained from female and male patients with and without HDV infection were used and HDV RNA was extracted on two different extraction platforms. On each platform, five HDV positive clinical plasma samples each comprising five 1.0 log_{10} dilution steps, five HDV negative clinical plasma samples that had tested negative for both hepatitis B surface antigen and HBV DNA, and a 1:100-dilution of the 1st WHO International Standard for HDV RNA containing a nominal concentration of 3.76 log_{10} IU/mL were extracted. Thus, 31 eluates were obtained and amplified twice accounting for 62 results. Additionally, four quantification standards with different concentrations and 2 negative controls were included in the run.

3.3 Instruments used in this study

3.3.1 The eMAG®

The eMAG® is a fully-automated nucleic acid extraction platform manufactured by BioMérieux S.A., Marcy l'Étoile, France (Fig. 6). This instrument allows parallel extraction of DNA and RNA out of primary sample tubes. It is a very flexible platform allowing extraction of various sample materials in parallel out of different primary devices including different input volumes within a single run. Magnetic silica particles and internal controls are added automatically. There are two independent working sections, which means that a total of 48 nucleic acid extractions can be done in parallel. Extracted nucleic acids can be eluted in different volumes. The platform is able to perform 48 extractions within 90 minutes (10).

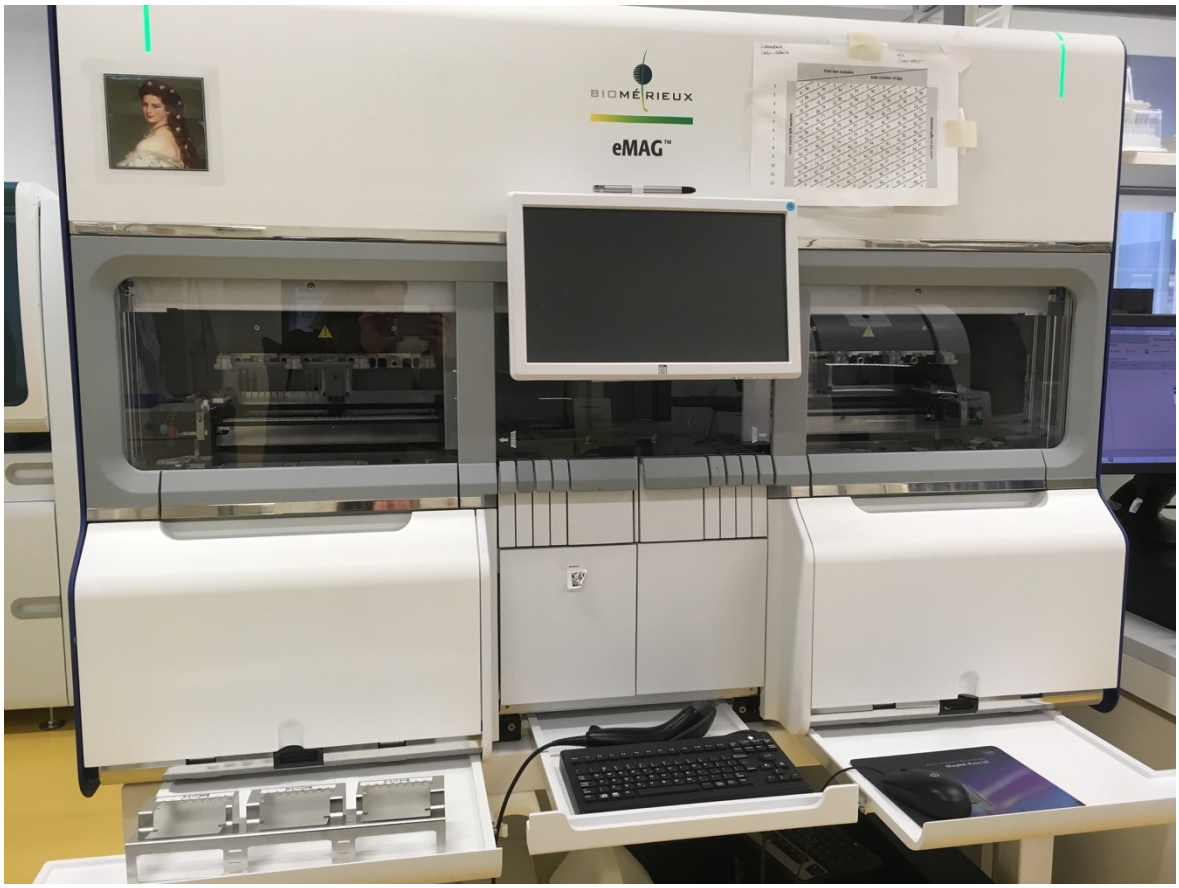

Fig. 6. The eMAG® platform (bioMérieux S.A., Marcy l'Étoile, France) in the Molecular Diagnostics Laboratory at the Medical University of Graz.

3.3.2 The MagNA Pure 24 System

The MagNA Pure 24 is a fully-automated clinical nucleic acid extraction system manufactured by Roche Diagnostic Systems, Rotkreuz, Switzerland (Fig. 7). It has been designed for extractions from 1 to 24 samples and input volumes ranging from 200 μL to 4 mL. Ten different types of samples can be extracted with this system including EDTA whole blood, serum, plasma, sputum, nasopharyngeal swabs, bronchoalveolar lavages, urine, cerebrospinal fluid, stool, and fresh or frozen tissue. Only one universal reagent kit, the MagNA Pure 24 Total NA Isolation Kit, is required (11).

Figure 8 shows the steps of nucleic acid purification on the MagNA Pure 24 System. With step one, samples are lysed, nucleases inactivated, and nucleic acids released. Then, magnetic glass particles are added allowing adsorption of the nucleic acids. After this, the separation of magnetic glass particles-nucleic acids complexes from the lysed sample (cell debris) is performed using a magnetic device. Then, washing steps are performed, each supported by a magnetic device holding back the complexes mentioned. With the last step, the elution buffer is added to the purified nucleic acids (11).

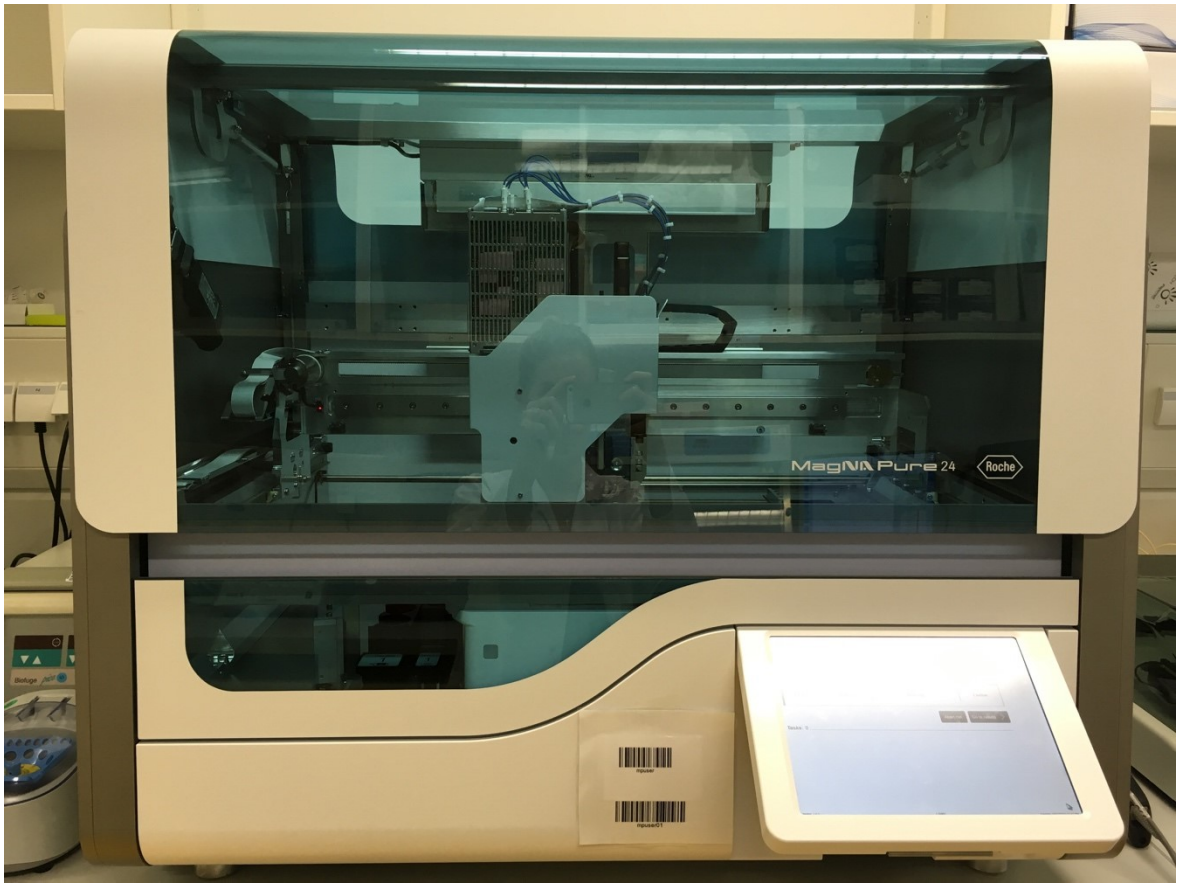

Fig. 7. MagNA Pure 24 (Roche Diagnostic Systems, Rotkreuz, Switzerland) in the Molecular Diagnostics Laboratory at the Medical University of Graz.

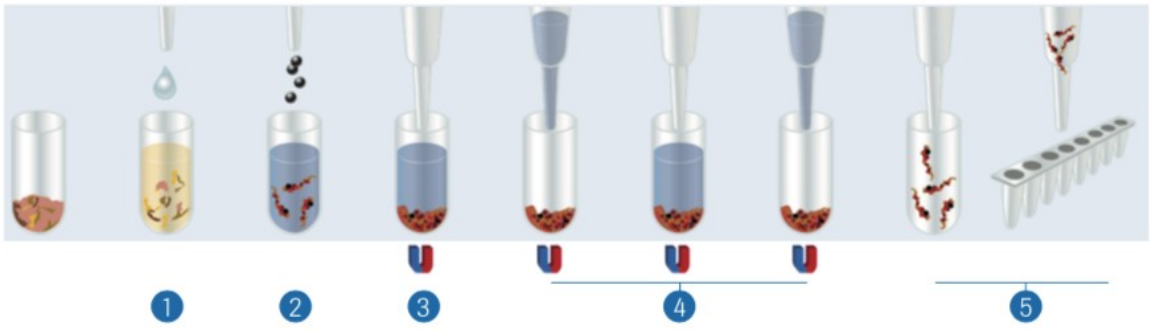

Fig. 8. Steps of nucleic acid purification (from: https://lifescience.roche.com/documents/MagNA_Pure_24_Brochure.pdf) (accessed 10.01.2019).

3.3.3 The LightCycler® 480 II

The LightCycler® 480 II is a plate-based real-time PCR amplification and detection instrument (Fig. 9). It contains a silver thermal block cycler unit and is operated by a separate desktop data station (12).

The system is able to reach and maintain reaction temperatures rapidly. Heating and cooling are performed using Peltier elements. A Therma-Base technology for optimal heat transfer and distribution to all samples is used. The Therma-Base relies on evaporation and condensation of a working fluid in a thin vacuum chamber enabling rapid heat distribution and temperature equilibration. Located under the Peltier elements, it ensures optimal heat distribution which leads to optimized well-to-well homogeneity and maximized inter-well assay reproducibility. One PCR run can be performed within 40 minutes, analyzing a 384-well block (12).

Fig. 9. LightCycler 480 II instruments in the Molecular Diagnostics Laboratory at the Medical University of Graz (figure kindly provided by Harald H. Kessler, Medical University of Graz).

3.4 Assays used in this study

3.4.1 Extraction assays

In this study, all samples were extracted using two different extraction protocols on two different automated nucleic acid extraction platforms, the eMAG® (bioMérieux S.A., Marcy l´Etoile, France) and the MagNA Pure 24 (Roche Molecular Diagnostics, Rotkreuz, Switzerland) (Table 2). Extracted nucleic acids were amplified and detected on the LightCycler 480 II (Roche) using the RoboGene® HDV RNA Quantification Kit 2.0 (Analytik Jena AG, Jena Germany).

Table 2
Extraction technology used in this study.

Extraction platform (manufacturer)	Extraction reagents used	Extraction protocol used	Sample volume (µL)	Elution volume (µL)	Extraction procedure
eMAG® (bioMérieux)	Specific eMAG® reagents	Generic	200	55	No proteinase K, lysis at room temperature (10 min), mixing[a], elution at 60°C
MagNA Pure 24 (Roche)	Total NA Isolation kit 1.0	Pathogen 200 hp 1.0	200	50	Proteinase K digestion, lysis at 65°C (time not available), mixing[a], elution temperature not available

[a] by pipetting up and down

In this work, amplification and detection of HDV RNA after extraction with the eMAG® platform has been designated *Method A*, while amplification and detection of HDV RNA after extraction with the MagNA Pure 24 platform has been designated *Method B*.

3.4.2 Amplification and detection assay

All HDV RNA extracts were amplified and detected with the RoboGene® HDV RNA Quantification Kit 2.0 on the LightCycler 480 II instrument. The RoboGene® assay has been designed for the quantification of HDV RNA extracted from human plasma samples by qPCR. The kit contains all components required for amplification and detection of HDV RNA (Fig. 10). With the RoboGene® HDV RNA Quantification Kit 2.0, all genotypes of HDV can be detected. By amplification of the included quantification standard strip, the concentrations of specimens can be determined. To exclude false-negative results, the whole assay including extraction is controlled by a synthetic internal control at a defined concentration included in the assay. For detection of amplification products and internal control, different fluorescent reporter dyes are used with labeled probes to measure the amplification of HDV RNA in samples and internal control (used as quantification standard) independently at different wavelengths (13). According to the manufacturer's package insert, the LOD of the RoboGene® HDV RNA Quantification Kit 2.0 was found to be 0.78, 0.90, or 1.15 $\log_{10}$ IU/mL after manual extraction using the INSTANT Virus RNA/DNA Kit (Analytik Jena) in combination with amplification and detection using the ABI 7500 Fast (Applied Biosystems, Darmstadt, Germany), Rotor-Gene™ 3000 (Qiagen, Hilden, Germany), or LightCycler® 480 instrument (Roche).

Fig. 10. HDV RNA Quantification Kit 2.0 (Analytik Jena) in the Molecular Diagnostics Laboratory at the Medical University of Graz.

The RoboGene® HDV RNA Quantification Kit 2.0 is based on the TaqMan® technology. This is a PCR technology useful for qPCR which is highly sensitive because it combines amplification with fluorescence-based online detection of the nucleic acid of interest called target (e.g. HDV RNA). This technology is based on a conventional set of target specific primers in combination with a fluorescence-labelled oligonucleotide probe, which is complementary to the desired target sequence (Fig. 11). When target and probe are mixed, the probe hybridizes with its target complementary sequence. The Taq DNA polymerase shows a 5`→3`exonuclease activity, which cleaves the probe and eventually displaces the fluorescent dye from the quencher. This increases the fluorescence signal, which is directly proportional to the target amplification during each PCR cycle (13).

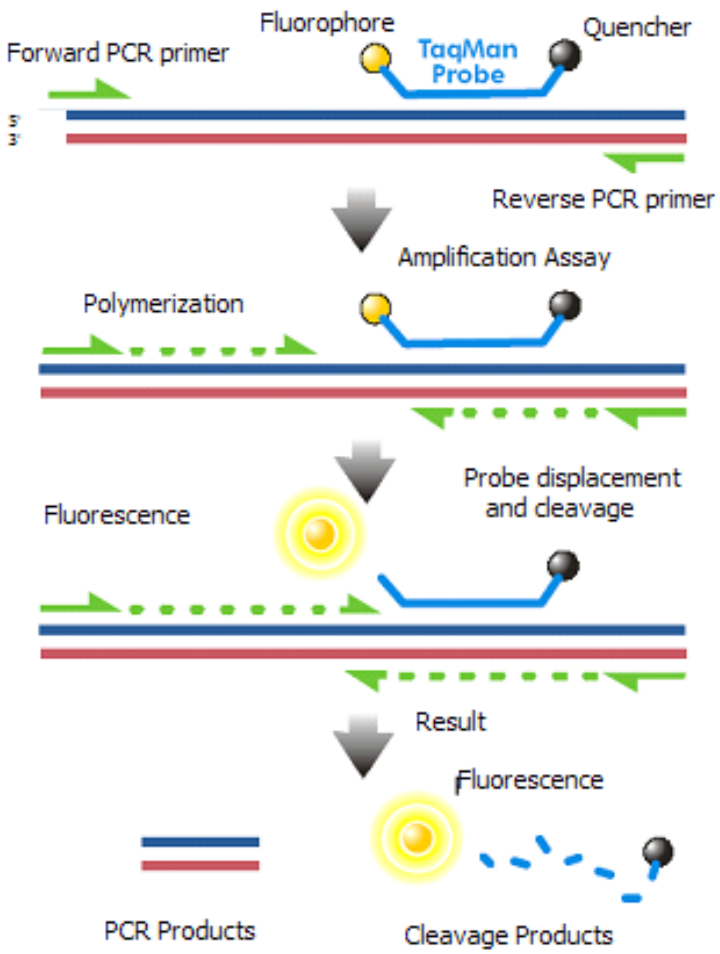

Fig. 11. A schematic illustration of the TaqMan® principle (from: https://en.wikipedia.org/wiki/TaqMan) (accessed: 22.01.2019).

4 Results

4.1 Analytical performance

4.1.1 Determination of the correction factor

The correction factor was determined for both methods with a 0.5 $\log_{10}$ dilution series starting with a concentration of 5.26 $\log_{10}$ IU/mL of the 1st WHO International Standard for HDV RNA. Ct values obtained are shown in Table 3, correction factors obtained in Table 4.

Table 3
Ct values and standard deviation (SD) of different dilutions. Each dilution was analyzed for three times.

Concentration [$\log_{10}$ IU/mL]	Ct values obtained by Method A (SD)	Ct values obtained by Method B (SD)
5.26	27.55 (0.29)	25.23 (0.38)
4.76	27.50 (0.44)	26.54 (0.41)
4.26	30.08 (0.23)	28.57 (0.41)
3.76	32.69 (0.91)	30.16 (0.48)
3.26	33.45 (0.34)	33.20 (1.24)

Table 4
Correction factors calculated according to formula shown in Figure 5.

Method	Correction Factor
A	922
B	190

4.1.2 Determination of the limit of detection

The limit of detection (LOD) was determined by analyzing a dilution series of the 1st WHO International Standard for HDV RNA starting with a dilution containing an HDV RNA concentration of 2.76 log_{10} IU/mL followed by 8 additional two-fold dilutions and one negative control (HDV negative plasma). Results are summarized in Table 5.

Table 5
LOD and upper and lower confidence thresholds per method.

Method	LOD [log_{10} IU/mL]	95% confidence interval
A	2.65	2.63 – 2.67
B	2.37	2.34 – 2.40

4.1.3 Accuracy testing

Accuracy testing was performed using members of the QCMD 2018 Hepatitis D Virus Program. Table 6 shows results obtained by extraction with either the eMAG® or the MP24 platform.

Table 6
Results obtained by accuracy testing utilizing the Quality Control for Molecular Diagnostics 2018 Hepatitis D Virus EQA Program 2018.

		Extraction with the eMAG®		Extraction with the MP24	
Sample Code	Consensus[a] (log_{10} IU/mL)	Result obtained	Log_{10} difference	Result obtained	Log_{10} difference
HDV 18S-01	2.03	TND	-	TND	-
HDV 18S-02	5.01	5.43	0.42	5.09	0.08
HDV 18S-03	3.05	2.76	0.29	2.87	0.18
HDV 18S-04	4.10	4.52	0.42	3.86	0.24
HDV 18S-05	4.11	4.45	0.34	4.01	0.1
HDV 18S-06	3.11	TND	-	2.31	0.8
HDV 18S-07	4.12	4.23	0.11	4.05	0.07
HDV 18S-08	4.06	3.97	0.09	3.64	0.42
HDV 18S-09	Negative	TND	-	TND	-

[a]Mean consensus calculated from data returned by participants with outliers removed.

TND, target not detected.

4.2 Clinical Performance

Of 25 HDV positive clinical samples, 15 tested positive with both of the methods, 11 of them within the analytical measuring range (Table 7). Three samples were detected below the LOD with both of the methods, one gave a positive result within the analytical measuring range with Method A but was detected below the LOD with Method B. Of the two discrepant results, HDV RNA was detected below the LOD with one of the methods while it could not be detected with the other method and vice versa. In 8 samples, HDV RNA was not detected with both of the methods. None of the 5 clinical plasma samples that did not contain HDV RNA tested positive with either of the methods.

In Table 8, values obtained from 11 HDV RNA-positive clinical samples that tested positive with both of the test systems within the analytical measuring range are shown. In four of them, the mean HDV RNA concentration exceeded 0.5 $\log_{10}$ when comparing the different test systems.

Table 7
Comparison of the results obtained from 25 clinical HDV RNA positive samples tested by Method A and Method B.

		Method B		
		Positive within analytical measuring range	DBLOD	TND
Method A	Positive within analytical measuring range	11	0	0
	DBLOD	1	3	1
	TND	0	1	8

DBLOD, detected below limit of detection; TND, target not detected.

Table 8
Results obtained from HDV RNA-positive clinical samples that tested positive with both of the test systems within the analytical measuring range.

Sample	Mean log_{10} (IU/mL) HDV RNA concentration obtained by Method A	Mean log_{10} (IU/mL) HDV RNA concentration obtained by Method B	Log_{10} difference between methods
1	6.70	5.90	0.80
2	5.76	4.92	0.84
3	4.82	4.46	0.36
4	3.63	3.46	0.17
5	5.97	5.53	0.44
6	4.93	5.07	0.14
7	3.87	3.81	0.06
8	4.23	3.74	0.49
9	3.21	3.10	0.11
10	3.92	3.34	0.58
11	3.27	2.43	0.84

5 Discussion

Quantification of HDV RNA is an essential tool to verify therapy success and to determine end of treatment. The RoboGene® HDV RNA Quantification Kit 2.0 is a CE-IVD-labeled kit for quantification of HDV RNA in human EDTA plasma or serum samples. According to the manufacturer package insert, the INSTANT Virus RNA/DNA Kit, a manual nucleic acid extraction assay, has been validated in combination with the RoboGene® HDV RNA Quantification Kit 2.0 including amplification and detection on LightCycler 480 II. However, in the majority of molecular routine diagnostic laboratories, automated extraction systems have replaced manual nucleic acid extraction assays in order to decrease hands-on time per sample and increase assay performance (14). When employing such an automated extraction system in combination with the RoboGene® HDV RNA Quantification Kit 2.0 on the LightCycler 480 II, the new test system must be validated by the user.

In a recent study, Bremer et al. compared four different automated nucleic acid isolation platforms for HDV viral load quantification with the manual INSTANT Virus RNA/DNA Kit (15). When HDV RNA was extracted with the COBAS AmpliPrep (Roche), the MagNA Pure 96 (Roche) or the QIAcube VRK (Qiagen), an underestimation of the HDV RNA concentration in serum (about 10-fold) was observed, while the QIAcube QBK (Qiagen) platform showed a slightly less underestimation (about 6-fold). To make results within different molecular routine diagnostic laboratories using different extraction and amplification/detection equipment comparable, it is of major importance to calculate correction factors for any combination.

In this study, determination of correction factors corresponding to the 1st WHO International Standard for HDV RNA for the test systems evaluated showed values of 190 and 922. It must be considered that the correction factor depends not only on the conversion from copies to IU but also on the combination of extraction and amplification/detection platforms including the particular sample equivalent used. This underscores the importance to estimate the individual correction factor when using a different laboratory setting than that validated and recommended by the manufacturer.

In this study, LODs determined for both test systems were found to be significantly (>1.0 log_{10}) when compared to those stated in the package insert. Extraction kit components such as silica bead surface and buffer chemistry may have an impact on the sensitivity. Also, a pathogen-related effect may be considered such as composition of viral envelope or association of RNA and proteins.

For accuracy testing, as expected, that member of the reference panel containing an HDV RNA concentration under the LODs of the test systems evaluated could not be detected. However, the member containing an HDV RNA concentration almost 0.5 log_{10} above the LOD of Method A could not be detected by that test system. Regarding HDV RNA-positive clinical samples, those containing the lowest HDV RNA concentration could not be detected by both or one of the tests systems. No false-positive results were obtained throughout the whole study. This confirms the superiority of automated nucleic acid extraction by reducing the probability of false-positive results due to contamination that vice-versa increases in relation to the number of manipulations involved by manual extraction procedures (16).

In conclusion, comparable results are of paramount importance for quantification of HDV RNA, especially for patients undergoing anti-HDV therapy in different centers. If using a commercially available kit, it is strongly recommended to follow the procedures validated by the manufacturer. When introducing a new extraction and amplification/detection combination, determination of the specific CF is mandatory to yield comparable results. With the 1st WHO International Standard for HDV RNA, the CF can easily be calculated for different laboratory settings leading to harmonization of quantitative results.

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