DiAGSure® Hepatitis D Viral Load Kit

Catalog No.: GDQ2354-96R

Pack size: 96 Tests

GCC BIOTECH

For use with: Agilent, Bio-Rad, Roche Lightcycler-96, Roche-Z480/Cobas-480, Applied Bio systems [ABI] 7500/QuantStudio 5/6, Thermo-Piko-Real, Rotor gene 5/6plex, Alta-96, Cepheid Real time PCR machines.

CE IVD | ISO 13485:2016 | ISO 9001:2015 | GMP Quality Good Manufacturing Practice Certification

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Background:

Hepatitis D is an inflammation of the liver caused by the hepatitis D virus (HDV), which requires HBV for its replication. Hepatitis D infection cannot occur in the absence of hepatitis B virus. HDV-HBV co-infection is considered the most severe form of chronic viral hepatitis due to more rapid progression towards hepatocellular carcinoma and liver-related death. The routes of HDV transmission, like HBV, occur through broken skin (via injection, tattooing etc.) or through contact with infected blood or blood products. Transmission from mother to child is possible but rare. Vaccination against HBV prevents HDV coinfection and hence expansion of childhood HBV immunization programmes has resulted in a decline in hepatitis D incidence worldwide. Chronic HBV carriers are at risk of infection with HDV. People who are not immune to HBV (either by natural disease or immunization with the hepatitis B vaccine) are at risk of infection with HBV, which puts them at risk of HDV infection.

Symptoms of HDV Infection:

In acute hepatitis, simultaneous infection with HBV and HDV can lead to a mild-to-severe hepatitis with signs and symptoms of indistinguishable from those of other types of acute viral hepatitis infections. These features typically appear 3–7 weeks after initial infection and include fever, fatigue, loss of appetite, nausea, vomiting, dark urine, pale-coloured stools, jaundice (yellow eyes) and even fulminant hepatitis. However, recovery is usually complete, development of fulminant hepatitis is infrequent, and chronic hepatitis D is rare (less than 5% of acute hepatitis). In a superinfection, HDV can infect a person already chronically infected with HBV. The superinfection of HDV on chronic hepatitis B accelerates progression to a more severe disease in all ages and in 70–90% of persons. HDV superinfection accelerates progression to cirrhosis almost a decade earlier than HBV mono-infected persons. Patients with HDV induced cirrhosis are at an increased risk of hepatocellular carcinoma (HCC); however, the mechanism in which HDV causes more severe hepatitis and a faster progression of fibrosis than HBV alone remains unclear.

Test Principle:

The HDV PCR assay can detect Hepatitis D virus and is a much more sensitive and fast detection method over conventional antibody-based and histopathological methods for the detection of viral infection or for describing the natural history of viral infection. The DiAGSure® Hepatitis D Viral Load Kit is an in vitro diagnostic (IVD) real-time polymerase chain reaction (qPCR) test involving TaqMan chemistry intended for the detection of Hepatitis D viral RNA in human clinical and viral culture samples. The kit can detect HDV genotypes 1-8. This two-plex PCR detects the Hepatitis D Structural antigen (HDAg) gene target of the viral genome and includes a heterologous internal control (IC) reference gene in a single-tube reaction.

Sample types:

Human Plasma, Serum, whole blood, tissue biopsy and other body fluids

Estimated operating time:

~1 hr 20 mins

Kit Contents:

Cap	Contents	Description	Volume	No. of vials
Red	UNG+OS Master Mix	Amplification Mix	60 µL	8
Amber	Primer-Probe Mix	Amplification Reagent	30 µL	8
Yellow	HDV Standard 1 (10^4 copies/µL)	Quantification Standard	100 µL	2
Yellow	HDV Standard 2 (10^3 copies/µL)	Quantification Standard	100 µL	2
Yellow	HDV Standard 3 (10^2 copies/µL)	Quantification Standard	100 µL	2
Yellow	HDV Standard 4 (10^1 copies/µL)	Quantification Standard	100 µL	2
Green	Internal Control (IC)	Heterologous Internal Control	500 µL	2
White	NFW	PCR-grade water	1 mL	1
Blue	Negative Control (NC)	Negative control	1 mL	1

Materials required but not provided

Reagents

- RNA extraction components: TrizinXP Viral RNA Isolation kit (from plasma/serum/body fluid).
- RNase free/sterile PCR grade water

Consumables

- Disposable tips containing hydrophobic filters
- Sterile RNase-free 1.5 ml vials (Eppendorf tubes)
- Suitable RNase-free 0.1 or 0.2 ml PCR tubes for use of RGQ instruments
- Suitable multiwell 96 plates (Roche, product code 04 729 692 001) for use of LC480 II instruments
- RNase-free Mic tubes and caps (BMS, ref 60653) for use on the Mic qPCR cycler
- Hard-shell® PCR plates 96-well, thin wall (Bio-Rad, Hsp9655) for use on the CFX96TM
- MicroAmp Fast Optical 96-well reaction plate 0.1 ml (Applied Biosystems, ref 4346906) for use on the QS5

Equipments

- Adjustable pipettes: 0.1-2 µl, 2-20 µl, 20-200 µl, 200-1000 µl
- Tube rack for 1.5 ml vials
- Cooling block (1.5 ml) or ice (for placing kit components during reaction set up)
- PCR cooling block or ice for PCR reaction tubes and 96-well plates
- Vortex mixer
- Benchtop centrifuge with a rotor for 1.5 ml tubes
- Centrifuge suitable for PCR plates
- Calibrated real-time PCR instrument
- Automated nucleic acid extraction system

Kit Stability:

- Kit is shipped on dry ice and should be stored immediately upon receipt at –20°C in a constant temperature freezer. Please refer to product label for final expiry date.
- Repeated thawing and freezing (>7x) should be avoided, as this may reduce sensitivity.
- If the reagents are to be used only intermittently, they should be frozen in aliquots.
- Shelf-life of the kit: 18 months from mfg. date

Specimen handling & Storage:

After sample collection, perform the test on the same day. Otherwise, store it in the following condition: 2 to 8˚C for no more than 24 hours. Storage condition is below -20 ˚C for no more than 3 days. Samples can be stored for a long time below -70 ˚C. Repeated freezing-thawing of samples should be avoided.

Transportation:

The foam box is sealed with ice for transportation.

Operation procedure

A. Sample preparation:

Clinical samples are extracted according to the corresponding requirements and steps with the viral RNA extraction kit, and the extracted RNA could be directly used for detection. During RNA isolation, add 5 µL of the Internal Control (IC) to the lysis buffer and proceed normally with the extraction steps. If the extracted RNA is not immediately tested after extraction, they can also be stored at -20°C and repeated freezing and thawing should be avoided. We recommend a starting sample volume of 200-500 µL and the elution volume to be 50-70 µL. It is advised to start amplification with at RNA of high purity (O.D.260/280 = 1.8-2.0). A starting RNA amount of 100-500 ng should be used. Too high RNA concentration might necessitate template dilution according to requirement. Note: Use of freshly extracted RNA is recommended.

B. Preparation of amplification reagent:

Take the reagents out from the -20˚C freezer and thaw them on ice. After thawing, briefly vortex the reagents followed by a short spin. The PCR reagents should be kept on ice.

C. TaqMan qPCR protocol:

Per sample, set up a single reaction according to the following table:

Components	Volume per reaction
UNG+ OS Master Mix	4 µL
Primer-Probe Mix	2 µL
Extracted RNA/ Standard/Negative Control (NC)	10 µL
NFW	4 µL
Total Volume	20 µL

***In case the Internal Control (IC) is not added during RNA isolation, it can be directly added to the master mix at 0.5 µL per reaction without any significant change in reaction volume.

D. Real-time PCR Instrument set up:

Set up the PCR tubes/strips/plate in the real time machine and label the sample slots accordingly.
Select the sample type (Unknown/PC/NTC) and select the acquisition channel for each slot as follows:

Gene	Channel	Source wavelength (nm)	Detection wavelength (nm)
HDV Gene	FAM (Green)	470	510
Reference gene (IC)	ROX (Orange)	578	604

E. PCR cycling conditions:

Step	Temperature (°C)	Time	No. of cycles
RT stage	50	10 min	**1**
Hold stage	95	3 min	1
PCR Stage	95	10 sec	45
	58	30 sec*	

*Acquisition step (FAM/ROX Channel)

F. Instrument compatibility:

The DiAGSure® Hepatitis D Viral Load Kit is compatible with a broad range of real-time PCR platforms. Performance of the kit has been verified on the following systems:

Agilent, Bio-Rad, Roche Lightcycler-96, Roche-Z480/Cobas-480, Applied Bio systems [ABI] 7500/QuantStudio 5/6, Thermo-Piko-Real, Rotor gene 5/6plex, Alta-96, Cepheid Real time PCR machines.

G. Performance Evaluation:

- **Specificity:**

The target sequences detected in this kit specifically targets the Hepatitis D Structural antigen (HDAg) gene of HDV. The primers used in the assay failed to give any amplification from RNA extracted from the blood of healthy individuals eliminating the possibility of non-specific annealing with control human RNA.

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MG711742.1      TGCCATCCGAGTGGACGTTCGTCCTCCTTCGGATGCCCAGGTCGGACCGCGAGGAGGTGG    615
OK142824.1      GCCATCCGAG-TGGACGTCTGTCCTCCTACGGATGCCCAGGTCGGACCGCGAGGAGGTGG    886
OK142936.1      GCCATCCGAG-TGGACGTCTGTCCTCCTTCGGATGCCCAGGTCGGACCGCGAGGAGGTGG    873
OK142863.1      GCCATCCGAG-TGGACGTTCGTCCTCCTTCGGATGCCCAGGTCGGACCGCGAGGAGGTGG    881
MH243745.1      GCCATCCGAG-TGGACGTTCGTCCTCCTTCGGATGCCCAGGTCGGACCGCGAGGAGGTGG    465
AB201271.1      GCCATCCGAGGTGGACGTTCGTCCTCCTTCGGATGCCCAGGTCGGACCGCGAGGAGGTGG    145
MT583803.1      GCCATCCGAGTTGGACGTCTGTCCTCCTTCGGATGCCCAGGTCGGACCGCGAGGAGGTGG    733
                  *   *    *******  ******** ******************************

MG711742.1      AGATGCCATGCCGACCCGAAGAGGAAAGAAGGATACGGACGCAAA-CCTGTGAGTGGAAG    674
OK142824.1      AGATGCCATGCCGACCCGAAGAGGAAAGAAGAACACGGACGCGAA-CCCGTGAGTGGAAC    945
OK142936.1      AGATGCCATGCCGACCCGAAGAGGAAAGAAGGACGCGAGACACGAACCCGTGAGTGAATT    933
OK142863.1      AGATGCCATGCCGACCCGAAGAGGAAAGAAGGACGCGAGACACGAACCCGTGAGTGAATT    941
MH243745.1      AGATGCCATGCCGACCCGAAGAGGAAAGAAGGACGCGAGACACGAACCCGTGAGTGGAAA    525
AB201271.1      AGATGCCATGCCGACCCGAAGAGGAAAGAAGGACGCG-GACGCGAACCTGTGAGTGGAAA    204
MT583803.1      AGATGCCATGCCGACCCGAAGAGGAAAGAAGGACGCGAGACACGAACCTGTGAGTGGAAA    793
                ******************************* *  **       * ** ******* *
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Alignment of Hepatitis D virus HDAg gene conserved locus

Furthermore, the primers did not cross-react with other related viruses like HAV, HBV, HCV, HEV, HIV-1, HSV-1/2, CMV, EBV, Adenovirus, Influenza virus A & B, Rhinovirus and COVID-19; as well as the most common bacteria like *E. coli, B. subtilis, Pseudomonas* sp. and pathogens like *Mycobacterium tuberculosis, Streptococcus pneumoniae, Vibrio cholerae, Chlamydia trachomatis*, and *Salmonella typhi*.

- **Linear range and LoD**

  The analytical sensitivity of the assay was obtained using a dilution series of quantified in-vitro transcribed HDAg RNA spiked in negative plasma sample as a template for detection. The linearity of the kit was observed in the range 10 – 10^8 RNA copies/µL. The Limit of Detection (LoD) of the kit is 25 RNA copies/mL under in-vitro conditions. The assay was performed in six independent replicates each in triplicate.

- **Precision**

Precision was calculated based on intra-assay and inter-assay variability of the lowest copy number kit quantitative standard (HDV Standard 4) for HDV detection (FAM channel).

Table 1. Intra and Inter-assay variability of HDV standard 4

	Mean Ct	Std. Dev	Coefficient of variance (%)
Intra-assay variability : HDV Standard 4	33.28083	0.18	1.6
Inter-assay variability : HDV Standard 4	33.64625	0.24	1.8

- **Reproducibility**

Reproducibility data permit a regular performance assessment of the DiAGSure® Hepatitis D Viral Load Kit as well as an efficiency comparison with other products. These data are obtained by the participation in established proficiency programs.

- **Safety information:**

The DiAGSure® Hepatitis D Viral Load Kit is for laboratory use only. Use proper safety measures while handling clinical samples, like wearing mask, gloves, lab-coat, etc.

- **C_t cut-off for each fluorescent channel:**

Target	Cut-off C_t	Interpretation
HDV gene (FAM)	Ct≤40	Viral RNA positive
IC (ROX)	Ct≤35	Internal control positive

- **Interpretation of the results:**

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid. If the controls are not valid, the patient results cannot be interpreted.

Refer to the table below for the validity and the interpretation of each specimen result according to the results of each channel.

Table 2. Interpretation of result

Target	HDV HDAg gene	IC	Interpretation	Results
Fluorophore*	FAM/ Green	ROX/Orange		
Case-1	Positive	Positive/ Negative	HDV Positive	HDV RNA is detected in the clinical specimen.
Case-2	Negative	Positive	HDV Negative	HDV RNA is not detected in the sample.
Case-3	Negative	Negative	Invalid	Testing should be repeated once from extraction. If a second failure occurs, it is reported to sender as invalid and sample recollection is recommended.

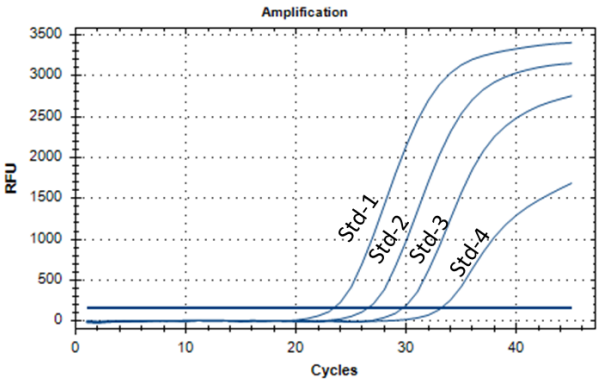
* If the target gene signal (FAM Channel) is strong, the IC (ROX Channel) may be negative.

- **Viral Load Estimation:**

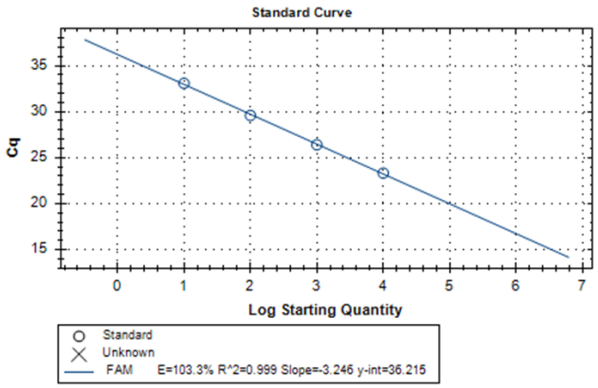
There are four quantification standards provided with the kit with viral RNA copies as follows –

Standard	Copies/µL
HDV Standard 1	10^4

HDV Standard 2	10^3
HDV Standard 3	10^2
HDV Standard 4	10^1



Amplification from HDV standards



HDV standard curve

A plot of Ct value vs RNA copies of the standards will fetch an equation wherefrom the viral copy number of an unknown sample can be deduced from its Ct value. The quantification standards exhibit linearity when Ct values are plotted against log (copies/ µL) with R^2 value>0.99. The efficiency of amplification should be above 80% with the slope of the standard curve in the range -3.1 to -3.8.

The quantitation standards are defined as RNA copies/µL. The following equation has to be applied to convert the values determined using the standard curve into copies/ml of sample material:

$$\text{Result (copies/ml)} = \frac{\text{Result (copies/µl) x Elution Volume (µl)}}{\text{Sample Volume (ml)}}$$

General precautions

Performing lab activities should always be done in compliance with general safety regulations. For more information on chemicals, consult the appropriate material safety data sheets (MSDS), which is part of the kit insert.

The following precautions should be taken to both avoid contamination and allow optimal performance and reproducibility of the assays:

- The PCR assay should only be performed by qualified laboratory personnel.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Physically separate the workplaces as outlined in table 3.

- Use disposable tips containing hydrophobic filters to prevent cross-contamination.
- Use RNase-free PCR vials.
- Thaw RNA samples always on ice and keep them on ice or on a cooling block.
- Keep enzymes always on ice or on a cooling block when taken out of the freezer. Handle enzymes with care and mix very gently.
- When thawed, spin down the reagents for 5 seconds in a centrifuge and mix by gently pipetting up and down.
- The cycling program should be programmed in the real-time PCR instrument before performing the assay.
- Spin down the PCR mixtures shortly in the PCR plate before transferring to the real- time cycler (not necessary for RGQ).
- Do not open the PCR vials/plates after PCR amplification.

Reagent storage and handling

Table 3. Handling procedures in different areas

Location	Handling
Area 1	UNG+ OS Master Mix, Primer-probe mix Preparation of PCR mix Aliquoting of PCR mix in respective wells Addition of Negative Control (NC)
Area 2	Storage of Quantitative Standards RNA extraction from samples Adding RNA-extracts to the real-time PCR mix Adding Standards to the real-time PCR mix
Area 3	Real-time PCR reaction

It is recommended to perform all activities in laminar air flow safety cabinet II in order to minimize the chance of cross-contamination.

Technical assistance:

Satisfaction of the customers is our utmost priority. For any kind of technical assistance, always feel free to reach out to us at tech.support@gccbiotech.co.in.

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GMP Certification

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ISO 13485:2016 Certification

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Manufacturer

In Vitro Diagnostic Medical Device