AMD Ltd Zena Max Hepatitis A Virus (HAV) qPCR Detection Kit

CE IVD

REF **KD764241-100**

Advanced Molecular Diagnostics Ltd is a diagnostics company specialising in the manufacture and supply of molecular biology instruments, reagents and consumables.

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Intended Use

The assay is an in vitro PCR reaction assay for the quantitative determination of purified Hepatitis A (HAV) viral RNA in human samples such as EDTA plasma, based on TaqMan® detection method with a highly sensitive one step RT-qPCR kit.

For *In Vitro* Diagnostic purposes only.

Overview

Acute viral hepatitis is one of the most prevalent infectious diseases worldwide, and hepatitis A, the causative agent for acute liver failure, hepatitis, is the most common form of acute viral hepatitis in much of the world[1]. The virus has cyclic epidemics and it is present across the world[2]. Hepatitis A (HAV) infections range from asymptomatic infections to fulminant hepatitis, and the disease is closely associated with unsafe water or food, inadequate sanitation and poor personal hygiene. The virus is primarily spread when an uninfected (and unvaccinated or previously infected) person ingests food or water that is contaminated with the faeces of an infected person[3].

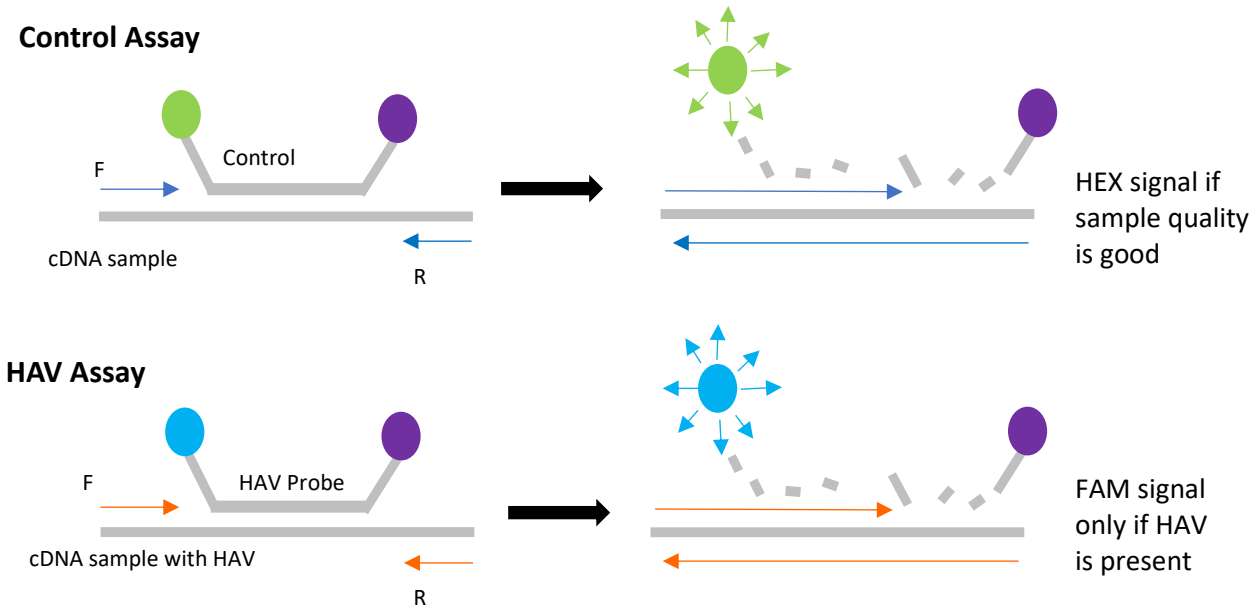
Principles of the Test

RT-qPCR (reverse transcription – quantitative PCR) has the ability to convert RNA into cDNA (complimentary DNA) through enzymes called Reverse Transcriptase. Quantitative PCR can be utilized for quantification of RNA, in both relative and absolute terms, by incorporating qPCR into the technique. This kit is designed for the identification of HAV using qPCR with hydrolysis probe detection.

This kit detects the presence of HAV using RT-qPCR by first reverse transcribing the genomic RNA target to cDNA, followed by amplification of the assay target and detection by the hydrolysis probe method of qPCR. The assay is designed to detect a highly conserved region of the HAV genome and consists of a forward primer, a reverse primer and a probe labelled with a 5' FAM™ reporter dye and a 3' quencher. As the new target cDNA strand is synthesized, the tightly bound probe is cleaved by the 5' to 3' exonuclease activity of Taq polymerase which releases the fluorescent reporter from the quencher and substantially increases the fluorescent signal. The point at which the fluorescence becomes detectable above the background, the quantification cycle (Cq), is proportional to the amount of target present in the sample. The lower the Cq, the greater the amount of target present. If, however HAV is not present, a FAM signal will not be produced. An internal positive control assay is provided in order to assess the quality of the isolated RNA and the effect of any PCR inhibitors that may be present. This assay contains two primers and a HEX labelled probe, designed to a highly

conserved region of human RNA and a positive signal indicates that the RNA quality in the sample is acceptable for diagnostic testing.

Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of HAV. The control assay in the master mix will produce a HEX signal if the cDNA quality is acceptable. The HAV assay will produce a FAM signal if the sample is positive, whereas negative samples will not



produce fluorescence in the FAM channel. Due to assay competition, the HEX signal may be reduced or absent when the FAM signal is strong.

Materials Provided

Kit Contents

Item	Volume
HAV qPCR Master Mix	2 x 1 ml
HAV standard 1 (1.0 x 10^8 copies/ml)	1 x 0.1 ml
HAV standard 2 (1.0 x 10^7 copies/ml)	1 x 0.1 ml
HAV standard 3 (1.0 x 10^6 copies/ml)	1 x 0.1 ml
HAV standard 4 (1.0 x 10^5 copies/ml)	1 x 0.1 ml
Nuclease-free water	1 x 1 ml

Reagent Storage and Handling

The kit should be transported and stored at temperatures between -18°C and -25°C. It will remain stable until the expiry date printed on the package if stored in the recommended

conditions. Repeated freeze-thawing of the kit components or exposure to light may reduce the performance of the assay. It is recommended that the master mix is aliquoted to avoid this.

Materials and Equipment Required (but not provided)

RNA Extraction: Leading brands of RNA isolation kit are acceptable for use with this diagnostic kit. If using any other kit, please validate for use with this assay before proceeding with sample testing.

PCR Instrument: This kit should be used with qPCR systems which can detect FAM and HEX fluorescent dyes. It is also compatible with both low and high ROX instruments

Consumables: AMD manufactures high quality nuclease and pyrogen free PCR plastic ware suitable for use with this kit. Use of other manufacturers' consumables is also acceptable.

Other Laboratory Equipment: Vortex, micro centrifuge, micro pipettes and tips, microfuge tube rack, PCR tube/plate rack, spectrophotometer.

Warnings and Precautions

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). Discard sample and assay waste according to your local safety regulations. It is essential to follow the instructions in this manual precisely, to ensure the best product performance.

Instrument compatibility

AMD HAV qPCR detection kit is compatible with the most common Real Time qPCR equipment with the capability of detecting FAM and HEX fluorescent dyes such as Biorad CFX96, Applied Biosystems 7500 Fast, QuantStudio 3,5,7, StepOne Plus, Aglient Mx3000, 3005P, Rotorgene Q, Cepheid Smartcycler, Analytik Jena qTower and Roche Lightcycler 480, 96.

Sample Collection, Storage and Transport

Whole blood can be either be collected in tubes containing EDTA for plasma and stored for a maximum of 6 hours at 2-8˚C before sample preparation. To prepare the sample for testing, separate the plasma from whole blood from clotted blood by centrifugation for 20 minutes at 800-1600 x g, then transfer to sterile polypropylene tubes. Plasma can be stored for up to 6 days at 2-8˚C. Aliquot and store the samples at -20˚C or -80˚C immediately if they are not to be used within this time-period. Freeze thawing may compromise the test results. Ensure that samples are stored correctly and kept away from any contamination.

For transportation, the samples should be placed in a shatterproof transport container to avoid the potential danger of infection due to sample leakage. Transport samples following the local and national instructions for the transport of pathogenic material, by courier, if possible, at a temperature of 2-8^{0}C and no longer than 6 hours following collection.

Assay Procedure

Sample Preparation

For optimal results use AMD LUCO Total RNA Extraction Kit (KD474882-50) to isolate the RNA from the sample. It is important to ensure that all samples are kept free from any contamination and correct storage procedures are followed to ensure there is no damage to the RNA. Store the RNA at 2-8°C for up to 24 hours, then at -20°C for longer term storage to ensure there is no damage to the RNA.

PCR Set up

1. Ensure that all reagents and samples are thawed completely, mixed and briefly centrifuged.
2. Aliquot the AMD HAV PCR master mix into separate tubes, ensuring to set up duplicate reactions of all samples and controls.
3. Set up the reactions using the table below.

Product	Volume
AMD HAV Master Mix	20µl
RNA Sample / Controls	5µl

4. Add the RNA samples and the HAV positive control to the PCR tubes/plate. Also add 5ul nuclease free water in place of the RNA as a No Template Control (NTC).
5. Briefly spin the PCR tubes or plate to ensure that the reagents are at the bottom and no air bubble are present.
6. Place the plate/tubes in the qPCR thermal cycler and use the following thermal profile:

Thermal Profile: Set the qPCR instrument to the stages below

Stage/Step	Temperature	Time
Stage 1: Step 1	55°C	15mins
Stage 1: Step 2	95°C	2mins
40 Cycles		

Stage 2: Step 1	95°C	10secs
Stage 2: Step 2*	58°C	30secs

*Data collection step in FAM (diagnostic assays) and HEX (internal control assay) channels.

7. When the run has finished, dispose of the PCR reaction tubes/plate in an appropriate manner in accordance with local and national regulations.

Data Analysis

8. Analyse the data if the software does not do this automatically at the end of the run. Export the data to Excel or a PDF report, depending on the qPCR instrument used, and view the results.

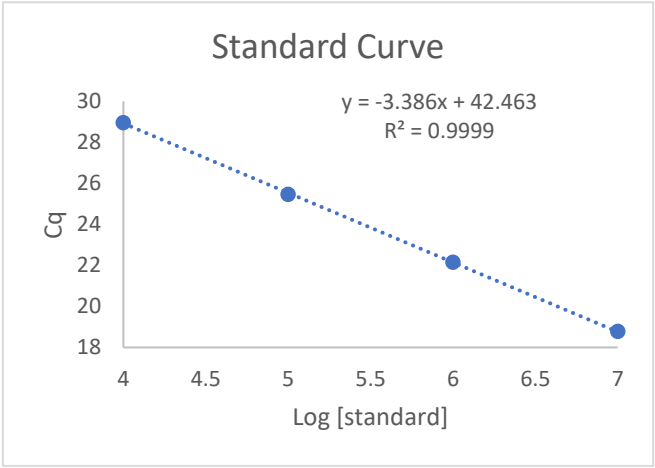


Figure 2. An example of the standard curve using the quantification cycles (Cq) of the four HAV standards. The equation can be used to quantify the amount of HAV, if it is present in the qPCR reactions.

Interpretation of Results

This is a quantitative assay that indicates the presence or absence of Hepatitis A Virus. The results should be interpreted as follows, using Table 1 as a quick reference guide:

- The internal control assay signal in the HEX (yellow) channel should be present but may be absent or have a high Cq value (low signal) when the diagnostic assay (FAM) signal is strong.
- If there is a signal in the FAM (green) channel, with or without a HEX signal, the sample is **positive** for HAV.
- If there is a HEX signal but no FAM signal, the sample is **negative** for HAV.
- If there is no signal in either channel, the result is **inconclusive**.

Table 1. Interpretation of the results obtained from the Zena Max HAV qPCR Kit.

Result		Interpretation
HEX	**FAM**	
Positive	Positive (Ct<38)	Positive for HAV
No Cq	Positive (Ct<38)	Positive for HAV
Positive (Ct<34)	No Cq	Negative for HAV
No Cq	No Cq	Inconclusive

Technical Specifications

Quality: All AMD kits manufactured under high quality standardization methods with unique precision and sensitive technology when compared by famous and approved diagnostic commercial Hepatitis A virus (HAV) assays.

Sensitivity: AMD Hepatitis A Virus (HAV) kit is a highly sensitive kit reaching up to 81 copies "rxn volume 25µl" under our validation methods and devices.

Specificity: AMD Hepatitis A Virus (HAV) kit is very specific up to 100% for Hepatitis A virus RNA under our validation methods and devices.

Data Analysis: Analyse the data if the software does not do this automatically at the end of the run. Export the data to Excel or a PDF report, depending on the qPCR instrument used, and view the results.

Product Limitations

This kit is for *in vitro* diagnostic procedures and should only be used by specifically trained laboratory personnel. The expiry date of all components must be checked before use and disposed of if expired. Occasionally mutations may arise in the genomic region targeted by the primers and probes of this assay, leading to reduction in performance or failure of the assay. The assay design and efficacy are reviewed periodically.

False Negative results may arise from several factors and their combinations including improper specimens as defined in 'collection, storage, and transportation methods' or degradation of the viral RNA during sample shipping and storage. The presence of RT-qPCR inhibitors or other types of interfering substances may also compromise the validity of results.

Additional Information

AMD produces real-time PCR kits with a wide range of applications for researchers from gene expression analysis, cDNA, and population genotyping studies, to the multiplex detection of several disease targets real-time PCR with excellent sensitivity and specificity. Please familiarise yourself with this document before using the AMD HAV PCR Kit.

References

1. Koff RS. Hepatitis a. The Lancet. 1998 May 30;351(9116):1643-9.

2. Forbes A, Williams R. Changing epidemiology and clinical aspects of hepatitis A. British Medical Bulletin. 1990 Jan 1;46(2):303-18.

3. Karayiannis P. Hepatitis B virus: virology, molecular biology, life cycle and intrahepatic spread. Hepatology international. 2017 Nov;11:500-8.

Contact

If you have any queries, comments or complaints please refer to our website at:

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Harmonised Symbols

The following is a key of all harmonised symbols used by AMD Ltd (Advanced Molecular Diagnostics) in Instructions for Use (IFUs) and product labelling.

Symbol	Definition	Details
	Manufacturer name and address	AMD Ltd BioCity Nottingham, Pennyfoot Street, Nottingham NG1 1GF United Kingdom
	Name and address of EU Representative	Advena Ltd Tower Business Centre, 2nd Floor, Tower Street, Swatar BKR 4013 Malta
	UDI-DI number for the product given	Basic: 506105998HAVGU UDI-DI: (01)05061059980533 UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-18 to -25 degrees Celsius
	Catalogue number	KD764241- 100
	Number of tests/reactions in this pack	100
	CE-IVD certified	According to Directive 98/79/EC