



AMD Ltd Zena Max Hantavirus qPCR Detection Kit

RUO



KD638928A-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

This assay is an in vitro reverse-transcription PCR (RT-PCR) test for the qualitative detection of Hantavirus RNA in human clinical specimens, such as whole blood (EDTA), plasma, or serum. The assay employs a high-sensitivity one-step real-time RT-qPCR method with hydrolysis probe detection.

This product is for in vitro use only.

Overview

Hantaviruses are a globally distributed group of rodent-borne, negative-sense, single-stranded RNA viruses capable of causing severe human disease. Human infection most commonly occurs through inhalation of aerosolised rodent excreta, including urine, faeces, and saliva, although transmission through rodent bites, scratches, or close contact with infected individuals has been reported for certain hantavirus species. Emerging and newly identified hantavirus strains continue to present a public health concern due to their potential for severe respiratory and renal disease and their capacity for geographic spread.

Clinical manifestations of hantavirus infection vary according to the viral species and geographic region but are generally associated with two principal syndromes: Hantavirus Pulmonary Syndrome (HPS) and Hemorrhagic Fever with Renal Syndrome (HFRS). HPS, reported predominantly in the Americas, typically begins with non-specific flu-like symptoms including fever, headache, myalgia, and fatigue, and may rapidly progress to severe respiratory distress, pulmonary oedema, and cardiopulmonary failure. HFRS, more commonly observed in Europe and Asia, generally presents as an acute febrile illness that may progress to thrombocytopenia, vascular leakage, haemorrhage, renal impairment, and, in severe cases, acute kidney failure [3;4]. Certain emerging hantavirus strains have additionally been associated with atypical clinical presentations and limited human-to-human transmission under close-contact conditions.

As hantavirus RNA may be transient and present at low concentrations during the early phase of infection, molecular detection using reverse-transcription quantitative PCR (RT-qPCR) provides a rapid and sensitive method for laboratory confirmation. Molecular testing is particularly valuable during acute infection and prior to seroconversion, when antibody responses may not yet be detectable. Early and accurate detection supports timely patient management, infection prevention measures, and epidemiological surveillance of emerging hantavirus infections.

Principles of the Test

The qPCR kit is designed for the qualitative detection of New Hantavirus (Andes virus) RNA by the real-time reverse-transcription Polymerase Chain Reaction (RT-qPCR) method. Detection of Andes virus is based on the amplification of a specific conserved RNA sequence and measurement of the amplification product during the PCR process using fluorophore-labelled probes. Presence of Andes virus RNA is indicated by fluorescence signal growth in the FAM fluorophore channel, while amplification of the internal control is detected in the HEX fluorophore channel.



The detection kit utilizes the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity. The ready-to-use Master Mix contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR reaction by amplification products. The kit is designed for in vitro diagnostics and provides qualitative detection.

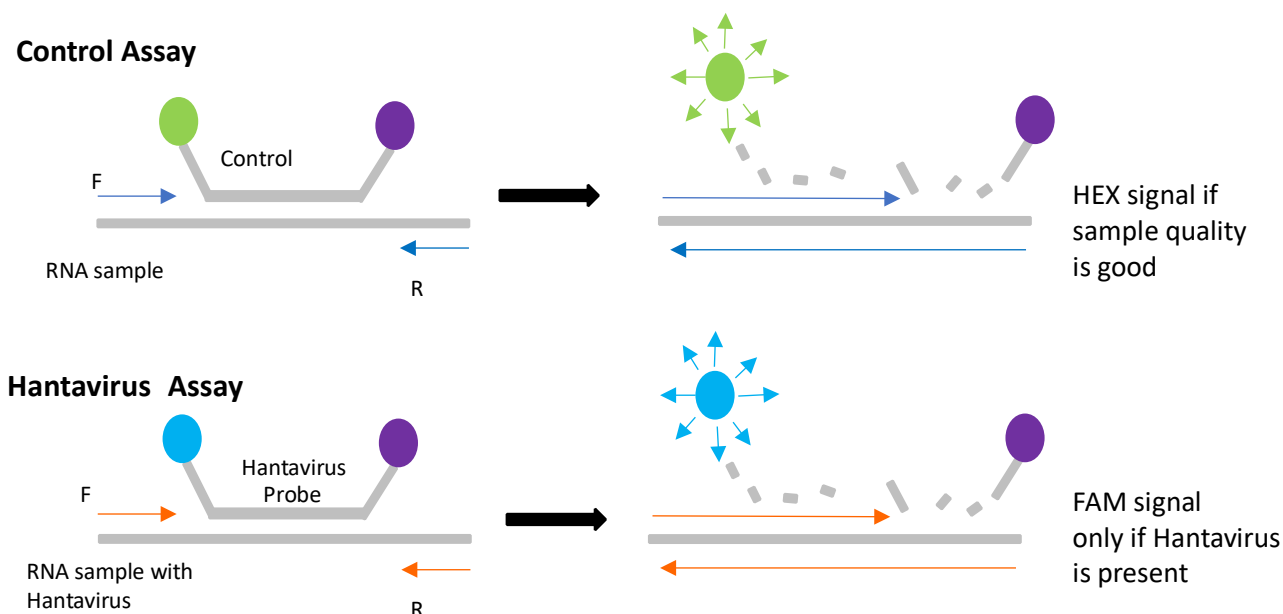


Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of Hantavirus. The control assay in the master mix will produce a HEX signal if the cDNA quality is acceptable. The Hantavirus assay will produce a FAM signal, if Hantavirus is present, however if it is not present, no FAM signal will be detected. Due to assay competition, the HEX signal may be reduced or absent when the FAM signal is strong.

Materials Provided

Kit content	KD638928A-100
Hantavirus qPCR Master Mix	2 x 1.0 ml
Hantavirus Positive Control	1 x 0.05 ml
Nuclease-free Water	1 x 1 ml

Reagent Storage and Handling

The kits should be transported and stored at temperatures between -18 °C and -25 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept. Repeated freezing and thawing of the kit components may result in lower detection quality. It is recommended that the master mix is aliquoted to avoid this. Avoid exposure to light. Ensure that all reagents are thoroughly thawed, mixed and pulse centrifuged before use.



Materials and Equipment Required (but not provided)

RNA Extraction: AMD manufactures the LUCO AMD NA Extraction Kit (KD474881-50) which can be used for isolating RNA from the samples. Other leading brands of IVD RNA extraction 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: AMD manufactures a high standard real-time qPCR system which is recommended for use with this kit. This kit can also be used with all other qPCR systems.

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.

Sample Collection, Storage and Transport

We advise using whole blood (EDTA), plasma or serum. Samples are stored for a maximum of 6 hours at 2-8°C before sample preparation. Please ensure that the sample is stored correctly and kept away from any contamination. 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°C and no longer than 6 hours following collection.

Assay Procedure

Sample Preparation

For optimal results use the LUCO AMD Nucleic Acid Extraction Kit (KD474881-50) to extract 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. Keep all reagents and samples on ice during this procedure.



2. Set up the reactions on a cool block or ice using the table below, ensuring to include duplicate reactions for all samples and controls. If preferred, make a mix of assay and reverse enzyme mix and aliquot 20µl into the PCR tubes/plate.

Product	Volume
AMD Hantavirus PCR Master Mix	20µl
RNA Sample	5µl
Total	25uL

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

Thermal Profile

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 assay) and HEX (internal control assay) channels.

6. 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

7. 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.

Interpretation of Results

This is a qualitative assay and the detection of Hantavirus in a sample is calculated by reference to the standard curve. The results should be interpreted as follows, using Table one as a quick reference guide:

- The internal control assay signal in the HEX channel should be present but may be absent or have a high Cq value (low signal) when the diagnostic assay signal is strong.
- If there is a FAM signal and positive HEX channel, the sample is **positive for Hantavirus**.
- If there is a HEX signal but no FAM signal, the sample is **negative** for Hantavirus.
- If there is no signal in either channel, the result is **inconclusive**.



Result		Interpretation
HEX	FAM	
Positive	Positive (Ct>38)	Positive for <i>Hantavirus</i>
No Cq	Positive (Ct>38)	Positive for <i>Hantavirus</i>
Positive (Ct>34)	No Cq	Negative for <i>Hantavirus</i>
No Cq	No Cq	Inconclusive

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

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.

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 the qPCR instrument before using the AMD RNA Hantavirus PCR Kit.

AMD PCR software offers several analysis modules, including quantification, melt curve, gene expression, allelic discrimination, and end-point analyses. A data file is automatically generated after a run with the gene expression module which allows you to easily view up to six different charts or tables, such as the amplification plot, standard curve, gene expression chart, plate layout, or melt peak with the Custom Data View tab. The software used will show screens including a Cq reading and the linearity of the samples used.

Contact

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

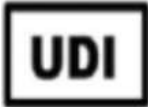
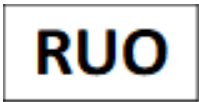
www.am-diagnostics.co.uk info@am-diagnostics.co.uk

References:

1. Centers for Disease Control and Prevention (CDC). About Hantavirus. Updated May 13, 2024.
2. JAMA — “What Is Hantavirus?” (Abdoler E.A., Malani P.N.). 2023.
3. Jonsson CB, et al. “Hantaviruses: A Global Disease Problem.” *Emerg Infect Dis.* 1997;3(2):95–104.
4. CDC. Clinical Overview of Hantavirus. Updated May 23, 2024.
5. Krüger DH, Schönrich G, Klempa B. “Human pathogenic hantaviruses and prevention of infection.” *Clin Microbiol Infect.* 2015. (via review of hantavirus diagnosis)

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
	UDI-DI number for the product given	Basic: 506105998HNVSDJ UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-18 to -25 degrees Celsius
	Catalogue number	KD638928A-100
	Number of tests/reactions in this pack	100
	RUO	