Instructions for use
ADI721-EHDV_NO_(EN)_V02
02/2025

EHDV REAL TIME

References: ADI721-100 & ADI721-500

Test for the detection of epizootic haemorrhagic disease virus (or EHDV) by real-time enzymatic gene amplification.
PCR test – 100 & 500 reactions

For veterinary *in vitro* use only

Sample	Individual analysis	Pooled analysis*
Blood	✓	✓
Organ (spleen and abortive tissues)	✓	✓

*Depending on the epidemiological situation, sample quality and country-specific guidelines (refer to these guidelines).

Included in the kit

Content		ADI721-100 kit 100 reactions	ADI721-500 kit 500 reactions
A5	Amplification solution	2 x 1 000 µL tubes with green cap (Ready-to-use reagent)	10 x 1 000 µL tubes with green cap (Ready-to-use reagent)
EHDV CTL+	Positive control EHDV	1 tube with purple cap (To reconstitute)	2 tubes with purple cap (To reconstitute)
PCR Buffer	CTL+ rehydration solution	1 x 1 000 µL tube with white cap (Ready-to-use reagent)	1 x 1 000 µL tube with white cap (Ready-to-use reagent)

Revision history

Date	Version	Change
11/2023	V01	Creation
02/2025	V02	Modification of "NF water" by "PCR Buffer". Addition of cervids and camelids species.

Note: minor typographical, grammar and formatting changes are not included in the revision history.

A. Introduction

Epizootic haemorrhagic disease (EHD) virus was first discovered in the United States in 1955. It affects mainly cattle and deer. There are seven different serotypes of the virus.

The virus is transmitted by biting midges of the genus *Culicoides*. The clinical signs of EHD in cattle are similar to those of bluetongue and include fever, anorexia, lameness, respiratory distress and potential death. Small ruminants can also carry the virus.

Early detection of the virus using RT-PCR helps to limit the spread of the disease before animals are moved to other regions or countries.

B. Test principle

The ADIAVET™ EHDV REAL TIME test is based on reverse transcription (RT) of RNA into complementary DNA. This reaction is followed by gene amplification of EHDV-specific DNA fragments. It detects simultaneously, in a single well:

- Epizootic haemorrhagic disease virus (FAM-labelled probe).
- GAPDH: internal control of extraction and amplification of specific endogenous DNA (HEX-labelled probe or its equivalent).

C. Storage conditions

On receipt, the kit must be stored at a temperature below -15 °C until its expiration date.
It is recommended to prepare aliquots of the A5 reagent if the number of tests requires it to be defrosted more than three times.
Do not defrost more than 3 times.
Store away from sunlight.
Do not mix reagents from different batches.

D. Additional material and reagents required but not supplied

- Real-time thermocycler with consumable.
- Instrument for homogenous mixing of tubes.
- 1-10 µL, 20-200 µL and 200-1 000 µL pipettes.
- Nuclease-free filtered micropipette tips.
- Nuclease-free microtubes of 1.5 mL and 2 mL.
- Powder-free latex or nitrile gloves.
- Nuclease-free water.
- Kit for nucleic acid extraction.

Additional kits for method adoption and PCR (U47-600)

- **Extraction Positive Control EHDV (Ref.: ADC72EPC).** Supplier reference material for method adoption that can also be used as a sentinel (Calibrated between 1 and 100x LOD_{Method})
- **LOD_{PCR} Positive Control EHDV (Ref. ADC72LD)** Confirmation of performance – Kit LOD_{PCR}.

E. Warnings and precautions

- For veterinary *in vitro* use only.
- For animal use only.
- For professional use only.
- All instructions must be read before performing the test and strictly respected.
- Do not use reagents after the kit expiration date.
- Do not use reagents if the packaging is damaged.
- Do not mix reagents from different batches.
- Do not open PCR wells or tubes after amplification.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.
- This kit contains products of animal origin. Certified knowledge of the origin and/or health status of the animals does not fully guarantee that the products do not contain any transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).

F. Nucleic acid extraction

1. Extraction kits

Nucleic acids must be extracted from the samples before using the kit. The DNA/RNA extraction kits listed below are recommended and supplied by Bio-X Diagnostics:

Product name	Extraction system	Number of tests and reference
ADIAMAG	Magnetic beads	200 tests: ref. NADI003 800 tests: ref. NADI003-XL

For extraction, consult the version of the package leaflet (available on the website) indicated on the certificate of analysis provided in the PCR kit used.

The validated extraction protocols are described in the kit's validation report. Other extraction kits may be used if validated by the user.

After extraction, nucleic acid extracts can be kept at +2/8 °C before use. For long-term storage, they must be kept at a temperature below -15 °C or -65 °C.

2. Controls

Use of controls is a means of verifying the reliability of the results. Controls are included per series of analyses according to the recommendations set out in the standards in force (Cf. AFNOR U47-600, etc.).

Controls	Validation of	Procedure
Control reagent (NTC)	Absence of amplification contamination	5 µl NF-Water in a well per PCR run
EHDV CTL+	Target amplification	5 µL of CTL+ in one well per PCR run
Extraction negative control	Absence of contamination for the extraction and amplification	1 extraction (water or lysis buffer) per extraction run
Extraction positive control	Extraction and amplification steps	1 extraction (positive sample between 1 and 100X LOD_{Method}) per extraction run

G. Procedure

1. Preparation of the CTL+ control

- Add **200 µL** of **« PCR Buffer »** per tube.
- Homogenize the tubes by suction and pressure, then use a mixer, such as vortex, > 20 seconds and until dissolution of the blue pellet.
- After reconstitution, aliquot and store the solution at a temperature below -15 °C until the kit expiration date. Do not thaw more than 3 times.

For each assay, use **5 µL** of denaturated CTL+ (see § « Denaturation of nucleic acid ») in one of the dedicated wells (see § « Amplification », Step 2).

2. Denaturation of nucleic acids

- For each extracted sample and each control, transfer at least 10 µL of nucleic acid to a tube or 96-well plate and store the rest at a temperature below -15 °C or -65 °C.
- Incubate for 3 minutes at +95 °C in a thermocycler or heating block.

- Immediately transfer the tubes or 96-well plates to ice or ice packs until use (avoids RNA renaturation).
- For use, see "Amplification", Step 2.

3. Amplification

Warning:

- Before starting, defrost the reagents at room temperature in the dark.
- Homogenise all reagents and samples before use.
- Store the reagents at a temperature below -15 °C after distribution.

Step 1: Distribute **20 µL** of the amplification reagent (A5) into each PCR well.
Step 2: Distribute **5 µL** of denatured nucleic acid extracts and **5 µL** of denatured controls into each dedicated well.
Use PCR Buffer for the Control reagent.
Step 3: Cover the wells with an appropriate optical film or caps.
Step 4: Start the PCR analysis.

The following programs have been developed for Applied Biosystems ABI Prism (7500, QuantiStudio5, Step-one, etc.), Agilent AriaMx Mx3000 and MX3005P, Roche Diagnostics LightCycler, Qiagen Rotor-Gene Q, Biorad CFX96 and Chromo 4 and BioMolecular System MIC equipment.

RNA standard program		FAST RNA program	
10 min. 45 °C		10 min 45 °C	
10 min. 95 °C		10 min 95 °C	
15 sec. 95 °C**	40 cycles	5 sec. 95 °C	40 cycles
60 sec. 60 °C*		30 sec. 60 °C*	

**30 sec. 95 °C for MX3000 and MX3005P.
* Reading and parameters for fluorescence acquisition:

Fluorochrome	Absorbance (nm)	Emission (nm)
FAM	494	520
HEX or equivalent	530	549
ROX	575	602

Note: The Quencher is non-fluorescent. The mixture contains a passive reference read in the same spectra as ROX for ABI Prism machines.

For other thermocyclers, please contact your sales representative or the customer relations department.

H. Interpretation of results

Display all curves and position the threshold line for each fluorochrome.

1. Test validation

Amplification is valid if the following results are obtained.
Expected Ct values (threshold cycle) values for CTL+ are indicated on the kit's certificate of analysis.

Controls	Amplification		Validation of
	FAM	HEX or equivalent	
Control reagent (NTC)	No	No	Absence of amplification contamination
EHDV CTL+	Yes	No/Yes	Target amplification
Extraction negative control	No	No	Absence of extraction contamination
Extraction positive control	Yes	No/Yes	Extraction and amplification steps

2. Interpretation of results

Nucleic acid extraction and amplification are **valid** for each sample if at least one typical amplification curve is observed in FAM and/or HEX or equivalent.

Amplification		Interpretation
FAM	HEX or equivalent	EHDV
No	Yes Ct < 33	Not detected
Yes, Ct < 34	Yes	Detected
Yes, 34 ≤ Ct < 40	Yes	Detected in small quantities
No	No/yes Ct ≥ 33	Not determined

"Detected in small quantities": The infection status cannot be defined: very recent infection (start of viraemia) or old infection (end of viraemia).
"Not determined": The results cannot be interpreted for the corresponding sample.
Possible causes:
Defective PCR due to inhibitors, set-up error, absence of samples, degraded samples and/or
Issue with nucleic acid extraction (loss or destruction of nucleic acids).
Recommendations:
Set up a new PCR assay using pure nucleic acid extracts and dilute to a 1/5 concentration in nuclease-free water;
If the test is still invalid, repeat the nucleic acid extraction by diluting the blood by half in PBS buffer. Finally, if the result cannot be interpreted, the sample cannot be used (presence of RT-PCR inhibitors; lysed or putrefied sample, etc.).

Symbols

Symbol	Meaning
REF	Catalogue number
	Manufacturer
	Upper temperature limit
	Use by date
LOT	Batch code
	Consult the instructions for use
	Content sufficient for "n" tests
VET	For veterinary *in vitro* use only – For animal use only
	Keep away from sunlight

Notes

1 Extract nucleic acids with

AdiaX Mag →

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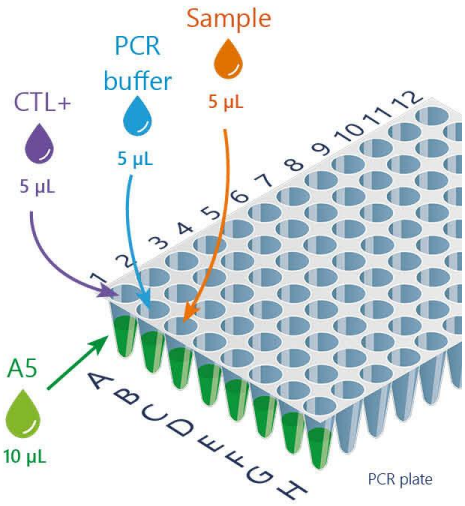
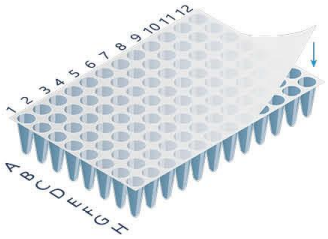
2 Distribute **20 µL** of **A5** amplification solution

3 Denature **10 µL of nucleic acids**, and each control **3 min at 95°C**.
Transfer immediately to ice or ice packs.

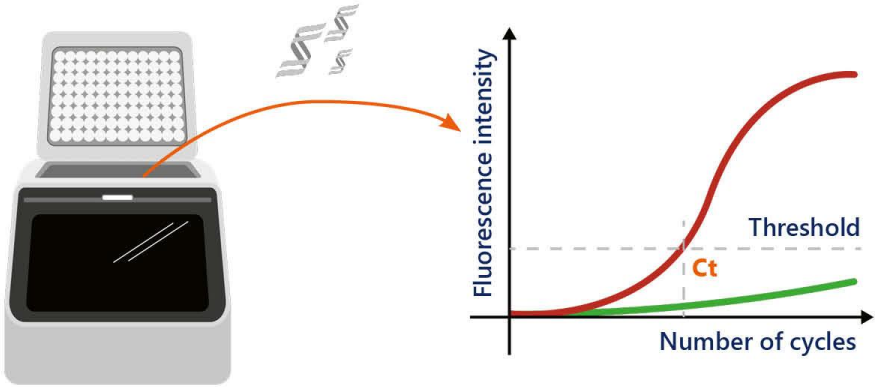
4 Distribute **5 µL** of denatured **nucleic acids**, denatured **CTL+** and **PCR buffer**.



5 Seal the wells



6 Start PCR analysis



*The notes do not replace the instructions for use of which they are a summary.

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