Adia X Lyo

Instruction manual
ADL73Y1_ORBI_NO_(EN)_V01
09/2024

BTV/EHDV

Reference: ADL73Y1-100 and ADL73Y1-500

Test for the detection of Bluetongue Virus (BTV) and Epizootic Haemorrhagic Disease Virus (EHDV) by real time enzymatic amplification

PCR Test – 100 and 500 reactions

For veterinary *in vitro* use only

Sample	Individual analysis	Pool of sample possible*, up to:
Blood	✓	✓
Organ	✓	✓

* Depending on the epidemiological case and on the quality of samples.

Kit composition

Content		ADL73Y1 Kit	
		100 reactions	500 reactions
A6	Amplification solution	1 lyophilized vial with blank caps (To reconstitute)	5 lyophilized vials with blank caps (To reconstitute)
Rehydration buffer	Rehydration solution	1 x 6 mL vial (Ready to use)	1 x 6 mL vial (Ready to use)
BTV CTL+	BTV positive control	1 tube with purple cap (To reconstitute)	2 tubes with purple cap (To reconstitute)
EHDV CTL+	EHDV positive control	1 tube with purple cap (To reconstitute)	2 tubes with purple cap (To reconstitute)
NF-Water	Nuclease-Free Water	1 x 1000 µL tube with white cap (Ready to use)	2 x 1000 µL tubes with white cap (Ready to use)

Revision history

Date	Version	Modifications
09/2024	V01	First version

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

A. Introduction

The bluetongue virus is a non-contagious viral arthropod-borne infectious disease due to an Orbivirus (family Reoviridae, virus ARN), mainly transmitted by hematophageous midges from Culicoides genus. More than 30 distinct serotypes exist inducing partial or no cross protections between them. Transmission by pregnant ewes has also been described. Transmission by contaminated blood injection is possible when needles and syringes are re-used. Samples for virus detection are bloods of animals with anticoagulants (EDTA). Virus is detected by isolation on embryonated eggs, *in vitro* cell culture, immunofluorescence on cell culture or by PCR.

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 also 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 these virus using RT-PCR helps to limit the spread of diseases before animals are moved to other regions or countries.

B. Test principle

ADIALYO™ BTV/EHDV test is based on the reverse transcription (RT) of RNA into complementary DNA. Then, cDNA is amplified with a DNA polymerase using specific primers of Bluetongue Virus (BTV) and Epizootic Haemorrhagic Disease Virus (EHDV). Both enzymatic reactions occur in the same tube (One-step RT-PCR). This test is intended to detect simultaneously, in one well:

- BTV (FAM labelled probe).
- EHDV (HEX labelled probe or its equivalent).
- GAPDH : internal control of extraction and amplification specific from an endogenous nucleic acid (Cy5 labelled probe).

C. Storage conditions

- Store the kit at a temperature below +2/8 °C after reception.
- Store away from sunlight and keep dry.
- After reconstitution, prepare aliquots and store them at a temperature below -15 °C until the expiration date.
- Do not thaw more than 3 times.

D. Material required but not provided

- Real-time Thermal cycler and device.
- Instrument for homogenous mixing of tubes.
- Pipettes of 1 - 10 µL, 20 - 200 µL and 200 - 1000 µL.
- Nuclease-free filtered pipette tips.
- Nuclease-free microtubes of 1,5 mL and 2 mL.
- Powdered-free latex or nitrile gloves.
- Nuclease-free water.
- Kit for nucleic acids extraction.

Additional kits for method adoption and PCR

- **Extraction Positive Control BTV (Ref.: ADC35EPC) or EHDV (Ref.: ADC72EPC).** Supplier reference material for method adoption that can also be used as a sentinel (Calibrated between 1 and 100xLOD_{Method}).
- **LD_{PCR} Positive Control – BTV/EHDV (Ref.: ADC73LD)** Confirmation of performances – LOD_{PCR} of kit.

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 expiration date.
- Do not use reagents if the packaging is damaged.
- Do not open PCR wells or tubes after amplification.
- Do not mix reagents from different batches.
- 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 sanitary state of the animals does not totally guarantee the absence of 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 acids extraction

1. Extraction kits

Nucleic acids must be extracted from the samples before using the kit. The RNA/DNA purification kits listed below are recommended 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 the extraction, consult the user manual version, available on the website, indicated on the certificate of analysis included in the PCR kit of interest.

Other purification kits can be used if they have been validated by the user.

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

2. Controls

Using controls allow to verify the reliability of the results. Controls can be included.

Control	Validation of	Usage
No Template Control (NTC)	Absence of amplification contamination	5 µL NF-Water in a well per run
BTV CTL+	BTV target amplification	5 µL BTV CTL+ in a well per run
EHDV CTL+	EHDV target amplification	5 µL EHDV CTL+ in a well per run
Negative extraction control	Absence of contamination for the extraction and amplification	1 extraction (water or lysis buffer) per run
Positive extraction control	Extraction and amplification	1 extraction (Positive sample between 1 et 100X LOD_{Method}) per run

G. Procedure

1. Amplification solution A6 preparation

- Add **1000 µL** of **« Rehydration buffer »** per A6 tube.
- Homogenize tube using a mixer, such as vortex, at least 20 seconds.
- After reconstitution, aliquot the solution and store at a temperature below -15 °C until the expiration date. Do not thaw more than 3 times.
- To use the A6, please refer to §« Amplification », Step 1.

2. Preparation of controls

- Add **200 µL** of **« NF-Water »** 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 CTL+ in one of the dedicated wells (see § « Amplification », Step 2).

3. Denaturation of nucleic acids extracts

- For each sample and control(s), transfer 10 µL of nucleic acids extracts in a tube or 96-plate and store the rest at a temperature below -15 °C or -65 °C.
- Incubate 3 minutes at +95 °C in a thermal cycler or heating block.
- Immediately transfer the tubes or 96-plate on melting ice or refrigerated block until use (to prevent RNA renaturation).
- To use, please refer to §« Amplification », Step 2.

4. Amplification

Warning:

- Before starting, rehydrate or thaw reagents at room temperature in the dark.
- Homogenize all reagents and samples before use.
- Store reagents at a temperature below -15 °C after use.

Step 1: Dispense **10 µL** of amplification solution (A6) per well.
Step 2: Dispense **5 µL** of denatured nucleic acids extracts and **5 µL** of denatured controls in each dedicated well.
Use NF-Water for the No Template Control (NTC).
Step 3: Cover the wells with an appropriate optical film or caps.
Step 4: Set up the amplification program.

The following program is defined for ABI Prism thermocyclers (like 7500, QuantStudio5, Step-one…) from Applied Biosystems, for Mx3000, Mx3005P and AriaMx from Agilent, for LightCycler from Roche Diagnostics, for Rotor-Gene Q from Qiagen, for CFX96 and Chromo 4 from Biorad and for MIC from BioMolecular System.

DNA/RNA Program	
10 min. 45 °C	
2 min. 95 °C	
5 sec. 95 °C	40 cycles
30 sec. 60 °C*	

The following program, compatible with ADIAVET™ BTV TYPE 1 REAL TIME (ADI391), BTV TYPE 3 REAL TIME (ADI711), BTV TYPE 4 REAL TIME (ADI541) and BTV TYPE 8 REAL TIME (ADI381) kits, has also been validated:

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

*Reading and parameters for fluorescence acquisition:

Fluorochrome	Absorbance (nm)	Emission (nm)
FAM	494	520
HEX or equivalent	538	554
Cy5	646	662
ROX	575	602

Note: The Quencher is non-fluorescent. The A6 solution contains a passive reference read in the same spectra as ROX for ABI machines.

For other thermal cycler instruments**,** please contact your sales representative or the customer relations department.

H. Reading and interpretation

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 (Threshold Cycle) values for the CTL+ are indicated on the certificate of analysis of the kit.

Controls	Amplification			Validation of
	FAM	HEX or equivalent	Cy5	
No Template Control (NTC)	No	No	No	Absence of amplification contamination
BTV CTL+	Yes	No	Yes/No	BTV Target amplification
EHDV CTL+	No	Yes	Yes/No	EHDV Target amplification
Extraction negative control	No	No	No	Absence of extraction contamination
BTV Extraction positive control	Yes	No	Yes/No	Extraction and amplification steps of BTV target
EHDV Extraction positive control	No	Yes	Yes/No	Extraction and amplification steps of EHDV target

2. Results interpretation

Nucleic acids extraction and amplification are **valid** for each sample if at least one typical amplification curve is observed in FAM, Cy5 and/or HEX or equivalent.

Amplification			Interpretation	
FAM	HEX or equivalent	Cy5	BTV	EHDV
No	No	Yes Ct < 33	Undetected	Undetected
Yes Ct < 34	Yes Ct < 34	Yes	Detected	Detected
Yes Ct < 34	No	Yes	Detected	Undetected
No	Yes Ct < 34	Yes	Undetected	Detected
Yes 34 ≤ Ct < 40	Yes 34 ≤ Ct < 40	Yes	Detected at low level	Detected at low level
Yes 34 ≤ Ct < 40	No	Yes	Detected at low level	Undetected
No	Yes 34 ≤ Ct < 40	Yes	Undetected	Detected at low level
No	No	No/Yes Ct ≥ 33	Undetermined	Undetermined

« Detected at low level »: The infection status can't be defined with certainty: either very recent or old infection (end of viremia).

« Undetermined »: no characteristic amplification curve.

Possible causes:
Defective PCR due to inhibitors, set up error, absence of samples, degraded samples and/or issue with nucleic acids extraction (loss or destruction of nucleic acids).

Recommendations:
Set up a new PCR assay using pure nucleic acids extracts and 1/5 dilutions in Nuclease-free water;
If the assay is inconclusive, perform a new nucleic acids extraction on blood diluted 1/2 in PBS 1x. If the results still can't be determined, the sample is declared unexploitable (RT-PCR inhibitors, degradation…).

Symbols

Symbole	Signification
	Catalog number
	Manufacturer
	Temperature limitation
	Use by
	Batch code
	Consult Instructions for Use
	Contain sufficient for "n" tests
	For veterinary *in vitro* use only – For animal use only
	Keep away from sunlight
	Keep dry

Notes

1 Extract nucleic acids with

Adia X Mag →

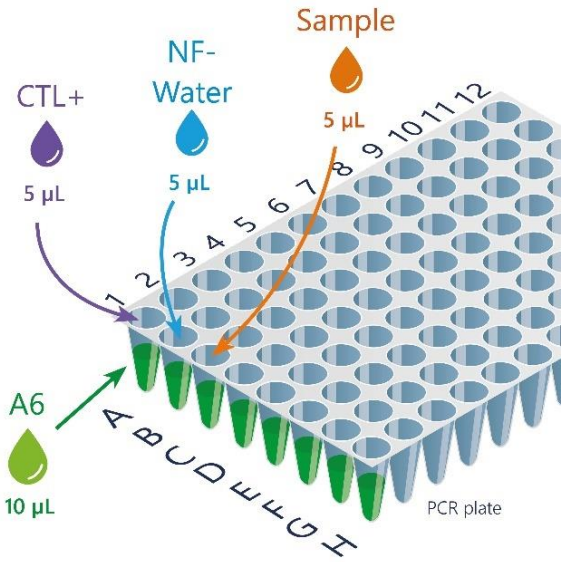
Scan me to discover Adiamag™

2 Add **1000 µL** of Rehydration buffer to the **A6** amplification solution

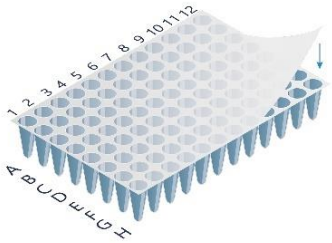
3 Distribute **10 µL** of **A6** amplification solution

4 Distribute **5 µL** of nucleic acids*, CTL+* and NF-Water

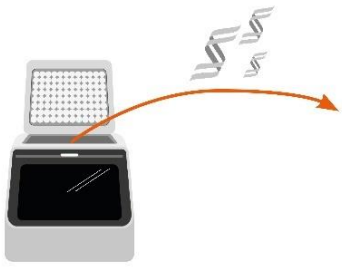
**Previously denatured 3 min. 95°C*

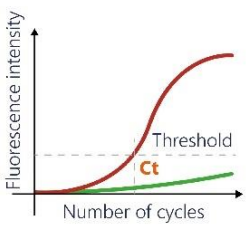


5 Seal the wells



6 Start PCR analysis





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

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