Adia X Vet

Instruction manual
ADI105_BVDV_NO_(EN)_V01
09/2022

BVDV REAL TIME

References: ADI105-100 & ADI105-500

Test for the detection of Bovine Viral Diarrhea Virus by real time enzymatic amplification
PCR Test – 100 & 500 reactions

For veterinary *in vitro* use only

Sample	Individual analysis	Pool of sample possible*, up to:
Blood/serum	✓	50
Ear notch	✓	25
Tissue (placenta, spleen, fœtal tissues…)	✓	✖
Milk	✓	Bulk

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

Kit composition

Content		ADI105 Kit	
		100 reactions	500 reactions
A5	Amplification solution	2 x 1000 µL tubes with green cap (Ready to use)	10 x 1000 µL tubes with green cap (Ready to use)
BVDV CTL+	BVDV 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)	1 x 1000 µL tube with white cap (Ready to use)

Revision history

Date	Version	Modifications
11/2020	NE105-14	Last version
09/2022	V01	Change to simplified leaflet format. Addition of ALLFLEX TST-L system preparation sample. Modification and addition of complementary kit references.

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

A. Introduction

Bovine Viral Diarrhoea Virus (BVDV), classical swine fever (CSFV) and border disease virus (BDV) in sheep are members of the pestivirus genus which belongs to the Flaviviridae family (like hepatitis C). BVDV, which induces mucosal disease in bovine, causes economic losses in cattle.

Many countries have started eradication programs of this disease, which involves a perfect management of infected animals. Indeed, those must be detected earlier with a high reliability. The earlier detection of these persistently infected animals is still necessary in eradication programs.

Most of these tests allow the detection of minimal quantities of BVDV in blood or organs of infected animals, even with less than three months old animals.

B. Test principle

ADIAVET™ BVDV REAL TIME test is based on the reverse transcription (RT) of RNA into complementary DNA. This reaction is followed by gene amplification of BVDV specific DNA fragments. This test is intended to detect simultaneously, in one well:

- BVDV, BDV and CSFV (FAM labelled probe).
- RNAse P internal control of extraction and amplification specific from an endogenous nucleic acid (HEX labelled probe or its equivalent).

C. Storage conditions

After reception, the kit should be stored at <-15 °C until the expiration date.

It is recommended to make aliquots of A5 solution as it should not be defrosted more than 3 times.

Do not thaw more than 3 times.

Store away from sunlight.

Do not mix reagents from two different batches.

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 BVDV EAR (Ref.: ADC10S02). BVDV positive ear notch sample for extraction control.**
- **Extraction Positive Control BVDV (Ref.: ADC10EPC). Supplier reference material for method adoption that can also be used as extraction control.**
- **LD_{PCR} Positive Control – BVDV (Ref.: ADC10LD) 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. Preparation of samples

Special case for ALLFLEX TST-L system:

In the special case of an ALLFLEX TST-L ear notch system containing a preservation buffer, 2 matrices can be used:

- Ear notch: biopsy without preservation buffer. In this case, remove the preservation buffer from the collection tube, before or after ejection of the biopsy. For extraction, use the biopsy as a matrix.
- Preservation buffer: Preservation buffer in which the biopsy has been incubated. In this case, eject the biopsy into the preservation buffer and adjust, if necessary, the volume to 250 µL with ALLFLEX preservation buffer and incubate for 1 hour at room temperature. For extraction, use the buffer as matrix.

2. 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
ADIAMAG LB3 buffer	Buffer for magnetic beads	125 mL: ref. NADI004
ADIAPURE TLB	Direct lysis	400 tests: ref. ADIADP10E1-400

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.

3. Controls

Using controls allow to verify the reliability of the results. Controls can be included by series of analysis according to the recommendations defined by the standards in force (Cf. AFNOR U47-600...).

Controls	Validation of	How to proceed
No Template Control (NTC)	Absence of amplification contamination	5 µL NF-Water in a well per run
BVDV CTL+	BVDV target amplification	5 µL 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. Use of CTL+

Add **200 µL** of **« NF-Water »** per tube.
Homogenize the tubes using 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).

2. 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 **20 µL** of amplification solution (A5) per well.
Step 2: Dispense **5 µL** of nucleic acids extracts or **5 µL** of 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: Start the PCR analysis.

The following programs are defined for ABI Prism thermocyclers (like 7500, QuantiStudio5, 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.

RNA standard programme		RNA FAST programme	
10 min. 45 °C		10 min. 45 °C	
10 min. 95 °C		10 min. 95 °C	
15 sec. 95 °C*	45 cycles	5 sec. 95 °C	45 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	538	554
Cy5	646	662
ROX	575	602

Note: The Quencher is non-fluorescent. The A5 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. 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 (Threshold Cycle) values for the CTL+ are indicated on the certificate of analysis of the kit.

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

2. Results interpretation

Nucleic acids 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	BVDV
No	Yes	Undetected
Yes	Yes	Detected
Yes	No	Detected
No	No	Undetermined

« 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 10x dilutions in Nuclease-free water.
If the assay is inconclusive, perform a new nucleic acids extraction.

Special case of ears notches in direct lysis with the ADIAPURE ™ TLB buffer:
Ear notches are usually analyzed in mixture.
This mixture may contain partially or completely inhibitory samples for the PCR reaction and mask positive result.
Thus, for direct lysis analysis with the ADIAPURE ™ TLB buffer, we propose the following interpretation and decision-making scheme.

Amplification		Interpretation & recommendations
FAM	HEX or equivalent	
No	Yes Ct<27*	Not detected
Yes	Yes/No	Detected
No	No	**Inhibited:** Mixture analysis: Test samples individually Individual analysis Test the pure and diluted sample (1/10)
No	Yes Ct>27*	**Potentially inhibited:** Mixture analysis: Test samples individually Individual analysis Test the diluted sample (1/10)

** The value of Ct of the internal control mentioned above may vary depending on the operating conditions. It must be defined for each laboratory.*

Symbols

Symbols	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

Notes

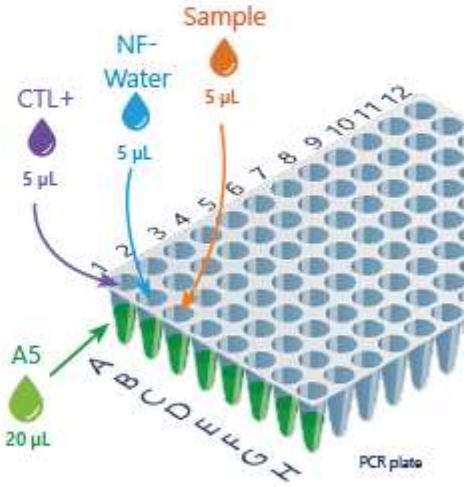
1 Extract nucleic acids with

Adia X Mag

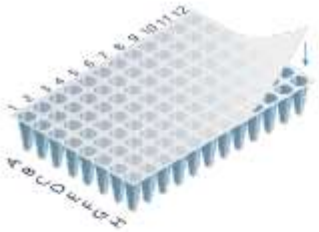
Scan me to discover Adiamag™

2 Distribute 20 µL of A5 amplification solution

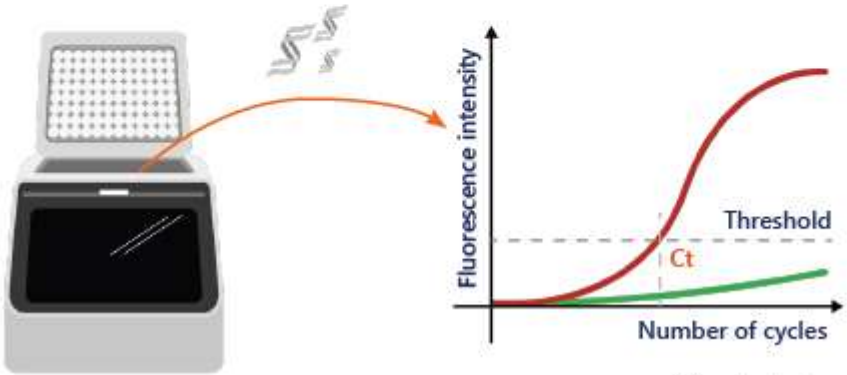
3 Distribute **5 µL** of nucleic acids, CTL+ and NF-Water



4 Seal the wells



5 Start PCR analysis



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

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