AdiaX Vet

Instruction manual
ADI581-VHSV_NO_(EN)_V01
01/2024

VHSV REAL TIME

Reference: ADI581-100

Test for the detection of viral hemorrhagic septicaemia virus by real time enzymatic amplification

PCR Test – 100 reactions

For veterinary *in vitro* use only

Sample	Individual analysis	Pool of sample possible*, up to:
Organ pool: spleen, kidney, heart or brain	✓	10 fishes
Genital products (sperm, egg, coelomic liquid)	✓	10 fishes
Culture supernatant	✓	✗

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

Kit composition

Content		ADI581-100 Kit 100 reactions
A5	Amplification solution	2 x 500 µL tube with green cap (Ready to use)
VHSV CTL+	VHSV positive control	1 tube with purple cap (To reconstitute)
EPC-Ext	Exogenous extraction control	2 tubes with yellow cap (Ready to use)
NF-Water	Nuclease-Free Water	1 x 1000 µL tube with white cap (Ready to use)

Revision history

Date	Version	Modifications
01/2021	NF581-02	II.4. Modification of PBS composition II.4. Addition of the ADIAMAG XL reference V. Applied thermocycler programming: quencher = none VI.2.B. Replacement of terms "positive" by "detected" and "negative" by "undetected".
01/2024	ADI581-VHSV_NO_(FR)_V01	New instruction template Addition of genital products (sperm, egg, coelomic liquid)

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

A. Introduction

Viral hemorrhagic septicaemia (VHS) is a major cause of mortality of rainbow trout in breeding. VHS is caused by the viral hemorrhagic septicaemia virus (VSHV), also known as the Egtved virus.

Brown trout, common shade, whitefish, and pike are susceptible to this virus, as well as marine species such as turbot and cod. Animals of all ages may be affected, but the disease is more common and more serious in juveniles. The acute form of the disease corresponds to the early stages of infection, with clinical signs as follows: rapid increase in mortality (can reach 100 %), lethargy, loss of equilibrium with sometimes spiralling swimming, haemorrhages at the base of fins, melanosis, anaemic gills, ascites and dilated abdomen, internal and external petechiae.

VHSV is a virus of the Rhabdovirus family and the subfamily of Novirhabdoviruses. It is a packaged virus, single-stranded negative RNA about 12 kb.

The reference diagnostic method has long been viral culture followed by identification by immunology or PCR. But this method requires an observation time of 3 weeks to certify the non-detection of viruses in the culture. The use of RT-qPCR allows to know the status of a breeding more quickly.

B. Test principle

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

- Viral hemorrhagic septicaemia virus (FAM labelled probe).
- Exogenous internal control (HEX labelled probe or its equivalent).

C. Storage conditions

On receipt, the kit should be stored at <-15 °C until the expiration date.
It is recommended to make fractions of A5 solution if it should be defrosted more than 3 times.
Do not thaw more than 3 times.
Store away from sunlight.
Do not mix reagents of 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 VHSV (Ref.: ADC58EPC).** Supplier reference material for method adoption that can also be used as a sentinel (Calibrated between 1 and 100xLOD_{Method}).
- **LD_{PCR} Positive Control – VHSV (Ref.: ADC58LD)** 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.

Extraction protocols are described in validation data. Other purification kits can be used if they have been validated by the user.

For the collection, transport and conservation of samples, refer to the recommendations of the Implementing Decision 2015/1554, and to the recommendations of the french national reference laboratory ANSES Ploufragan-Plouzané-Niort (REF: ANSES/PLOU/MA/3).
After analysis, the samples or residues as well as the extracted nucleic acids are kept at a temperature below -15 ° C for at least 1 month.
Extracted RNAs are sensitive molecules. The extraction is performed at room temperature and must therefore be as fast as possible to avoid damage. The extracted RNAs can be stored at the end of the extraction on ice or at +2/8°C for a few hours, then should be stored at <-65°C.

2. 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
VHSV CTL+	VHSV 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 EPC-Ext

EPC-Ext must be added to each sample and extraction controls.

- Aliquot and store the solution at a temperature below -15 °C according to the size of extraction series. Do not thaw more than 3 times.
- Add **5 µL** of EPC-Ext in the first nucleic acids extraction lysis buffer.

2. 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).

3. 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 (A5) per well.
Step 2: Dispense **5 µL** of nucleic acids extracts and **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, 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.

RNA standard program	
10 min. 45 °C	
10 min. 95 °C	
15 sec. 95 °C*	45 cycles
60 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
VHSV CTL+	Yes	Yes/No	Target amplification
Extraction negative control	No	Yes	Absence of extraction contamination
Extraction positive control	Yes	Yes	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	VHSV
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.

Bibliography

- Woah 2021. Chapiter 2.3.5 : Infection with infectious haematopoietic necrosis virus [https://www.woah.org/fileadmin/Home/eng/Health_standards/aahm/current/2.3.05_IHN.pdf]
- REGULATION (EU) 2016/429 of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law') [https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32016R0429]
- REGULATION (EU) 2020/689 of 17 December 2019 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for surveillance, eradication programmes, and disease-free status for certain listed and emerging diseases [https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020R0689]
- Diagnostic methods and procedures for the surveillance and confirmation of infection with VHSV and VHSV v2021.2 [https://www.eurl-fish-crustacean.eu/news/nyhed?id=33e113ac-a3e1-4820-b64f-cda52d289c00]
- Détection du virus de la Nécrose Hématopoïétique Infectieuse (vNHI) par RT-PCR en temps réel par le Laboratoire de Ploufragan-Plouzané-Niort, laboratoire national de référence pour les maladies règlementées de poissons. RÉF : ANSES/PLOU/MA/3

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

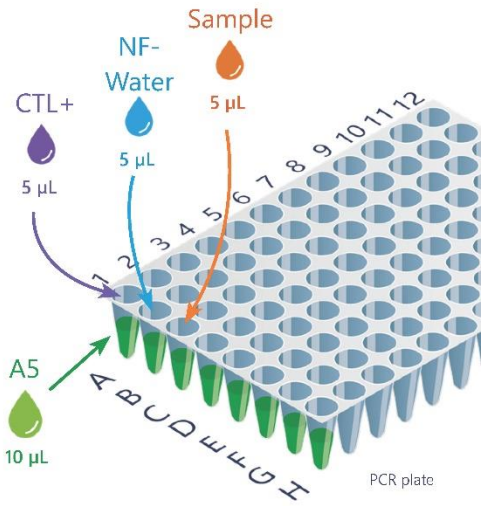
1 Extract nucleic acids with

AdiaX Mag

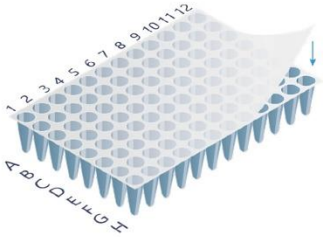
Scan me to discover Adiamag™

2 Distribute **10 µL** of **A5** amplification solution

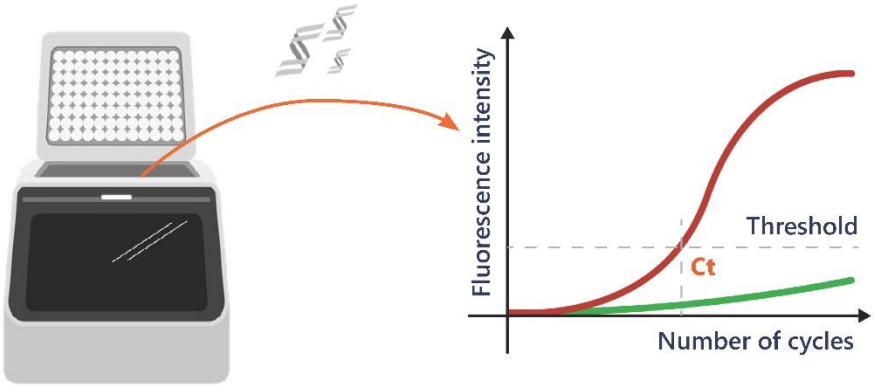
3 Distribute **5 µL** of nucleic acids, **CTL+** and **NF-Water**



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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