INSTRUCTION MANUAL

ADIAVET™ ASFV FAST TIME

TEST FOR THE DETECTION OF AFRICAN SWINE FEVER VIRUS BY REAL-TIME ENZYMATIC GENE AMPLIFICATION (PCR TEST)

References:
ADI551-50 (50 reactions)
ADI551-100 (100 reactions)

VET

English version
NE551-02
2019-07

ADIAVET™ ASFV FAST TIME

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Revision historic

N/A	Not Applicable (first publication)
Correction	Correction of document anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

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

Release Date	Part Number	Change type	Change summaru
2018-10	NE551-01	N/A	First publication
2019-07	NE551-02	Technical change	Addition of a "NF-Water" tube in the kit. Add protocol QIAamp® Viral RNA. Analysis of pool of samples. Change of the ADIAMAG reference.

I. General informations

1. Purpose of the test

ADIAVET™ ASFV FAST TIME kit allows to detect the African Swine Fever virus (ASFV) through real-time Polymerase Chain Reaction (PCR) technology on samples from pigs or wild boars.
Samples can be:

- liquid matrices: EDTA blood, serum, cell culture supernatant.
- lymphoid organs
- swabs from blood or exudate

2. Pathogen

African swine fever is a highly contagious hemorrhagic disease that affects pigs, warthogs, wild boars from Europe and America. All age groups are susceptible to the disease. The pathogen responsible for ASF is a double-stranded DNA virus of the Asfarviridae family. The virus is transmitted among suidae through direct contact or by ingestion of contaminated meat products. Soft ticks of the genus Ornithodoros can also transmit the virus but are not obligatory vectors. In its highly virulent forms, African swine fever is characterized by high fever, loss of appetite, haemorrhages in the skin and internal organs; death occurs in 2 to 10 days on average. Mortality can reach 100%.
Clinically, it is difficult to differentiate infections caused by classical swine fever virus from those due to the ASFV virus, hence the need for a differential diagnosis in the laboratory is request. PCR is a highly sensitive and rapid tool for ASFV detection and differentiation from the CSFV.

3. Description and purpose of the test

This test is based on enzymatic gene amplification or PCR technology.
Amplified products are detected in real-time thanks to a specific labelled hydrolysis probe (5'-exonulease technology).

The ADIAVET™ ASFV FAST TIME kit enables the simultaneous detection of:

- ASF virus (probe labelled in FAM),
- RNaseP, an internal control of extraction and amplification steps specific from an endogenous DNA (probe labelled with a fluorochrome read in the same spectra as VIC and HEX).

Adiagene recommends using this test with DNA purification kits (Qiagen and Macherey-Nagel). Other purification kits can be used if they have been validated by the user.

This kit was designed to allow a differential diagnosis between ASFV and CSFV. Common extraction protocols for the detection of CSFV and ASFV can thus be used from blood, serum, cell culture supernatant, swab, and tissue.

Analysis options according to the specimen:

Specimen	Individual analysis	Pool of samples is possible*, up to
Tissue (spleen, tonsil, ganglion…)	☑	10
Whole blood, serum, cell culture supernatant	☑	20
Swab of blood or exudate	☑	☑

* It depends on the epidemiological case and on the quality of the specimen. ASF is a disease regulated by OIE. You must refer to National control plan and agreement.

II. Material & reagents

1. Reagents provided with the kit

REF ADI551-50		
A5	Amplification solution	1 x 1000 µl tube with green caps (Ready to use)
ASFV CTL+	Positive control ASFV	1 tube with purple caps (To reconstitute)
NF-Water	Nuclease-free Water	1 x 1000 µl tube with blank caps (Ready to use)
REF ADI551-100		
A5	Amplification solution	2 x 1000 µl tube with green cap (Ready to use)
ASFV CTL+	Positive control ASFV	1 tube with purple caps (To reconstitute)
NF-Water	Nuclease-free Water	1 x 1000 µl tube with blank caps (Ready to use)

Package insert downloadable from www.biox.com

2. Validity and storage

On receipt, the kit should be stored at <**-15°C**.

It is recommended to make fractions of A5 solution if it should be defrosted more than 3 times.

Do not defrost reagents more than 3 times.

Real time reagents are susceptible to light: store them in the darkness.

The A5 reagent is ready to use for PCR reaction.

Do not mix reagents of two different batches.

3. Use of ASFV CTL+

ASFV CTL+ is a positive control of amplification.

Add **200 µl "NF-Water",** included in the kit, to the ASFV CTL+ tube and vortex at least 20 seconds.

Aliquot this solution by 6 or 12 µl and store them to <-15°C.

For each analysis, use 5 µl of ASFV CTL+ in a well.

4. Equipment required but not supplied by Adiagene

Material should be Nuclease-free (e.g. autoclaved 25 minutes twice at +120°C or once 60 minutes at +121°C)

- Thermal cycler with consumables for real-time PCR: 0.2 ml PCR tubes or closed 96-wells PCR plates with optical quality.
- Class II Microbiological Safety Cabinet
- Centrifuge for microtubes
- Universal laboratory mixer mill
- Etuve, heating baths or block heaters
- Vortex
- 1 - 10 µl pipette, 20 - 200 µl pipette and 200 - 1000 µl pipette
- Nuclease-free filter tips
- Nuclease-free microtubes: 1.5 ml and 2 ml
- Latex or nitrile powder-free gloves
- Metal beads 3 mm
- Scalpel blades
- 96-100% ethanol solution
- Nuclease-free water
- Sterile saline water (NaCl 8.5 g/l)
- PBS 1X buffer (pH7.4)

- Extraction kit (individual silica columns)

- QIAamp® DNA Mini Kit (Qiagen, 50 tests: ref. 51304 or 250 tests: ref. 51306) or DNeasy Blood & Tissue Kit (Qiagen, 50 tests: réf. 69504 or 250 tests: réf. 69506)
- NucleoSpin® RNA Virus (Macherey-Nagel, 50 tests: réf.740956.50 or 250 tests: réf. 740956.250)
- QIAamp® Viral RNA (Qiagen, 50 tests: réf. 52904 or 250 tests: réf. 52906).

or

- Automated DNA/RNA extraction kit using magnetic beads

- ADIAMAG (Bio-X Diagnostics: réf. NADI003), 200 tests.

III. Recommendations before the analysis of samples

Before starting the test, read the entire protocol and scrupulously respect it.

1. Precautions

Adiagene has validated this PCR test with the use of Bio-X Diagnostics, Qiagen and Macherey-Nagel extraction kits. Other extraction kits can be used with a previous validation.

Follow the supplier's instructions for the storage, the preparation and the use of the extraction reagents.

Some kits include and/or need the use of toxic reagents. These reagents should be handled with gloves and into a chemical cabinet.

We strongly recommend that only appropriately trained personnel perform this test. Ensure the accuracy and precision of the micropipettes used. The quality of the obtained results depends upon rigorous respect of good laboratory practices.

The PCR generates large amount of amplified DNA. A few molecules of amplified products are sufficient to generate a positive result. It is important to reserve 2 rooms, one for manipulation of samples to be tested, and another one for amplified products analysis. Do not open the PCR tubes after amplification.
Samples for analysis should be handled and disposed of as biological waste. **Take all measures of security and confinement required for the manipulation of the concerned biological agents.**

We recommend using fractions of demineralised and saline water and to autoclave them 25 minutes at +120°C. Take a new fraction for each new manipulation to avoid contamination.

2. Storage of samples and DNA extracts

Samples can be stored several days at +2/8°C, several months at -15°C after acceptance by the analytical laboratory.
However, it is up to the laboratory to ensure the state of conservation of the sample according to the time since the death of the animal, as well as conditions of transport and storage of samples.

Extracted DNAs are sensitive molecules. The extraction is performed at room temperature and must therefore be as fast as possible to avoid damage. The lysates can be stored on melting ice at the end of the extraction or at + 2/8 °C for a few hours and then stored at <- 15 °C.

3. Controls preparation

The use of controls allows verifying the reliability of the results.
The controls are included per trial of analysis. A trial is defined as all the samples treated in the same conditions.

All the steps of the analysis procedure (extraction+amplification), for all the types of samples, are validated with the association of the controls included in the Adiagene kit.

- The internal endogenous control (RNase P) naturally found in the samples verifies the extraction and amplification steps of each sample.

- The ASFV CTL+ valides the amplification of the both targets.

Other controls must or could be added:

- **Negative control of extraction (required)**

To verify the absence of cross-contamination, at least one negative control must be included per trial. The control is a negative sample, for example a buffer used for dilutions.

- **Positive control of extraction (recommended)**

A positive control could be added in each trial. It could come from a ASVF positive sample available in the laboratory or from a negative sample spiked with a ASFV solution. This positive control should be closed to the limit of detection of the method. It will inform about the fidelity of the obtained results between different trials.

IV. Extraction and Purification

All the centrifugations are performed at 18-25°C.

1. Extraction using QIAamp® DNA Mini kit or DNeasy ® Blood and Tissue.

	Blood, serum, cell culture supernatant	Tissue	Swab
Preparation of the sample	**100 µl** (individual or pool)	**20-30 mg** (individual or pool) **+300 µl** of **PBS 1X.**	Add **1 ml** of **PBS 1X.** Vortex
		Add **1 metal bead** in the microtube. Disrupt 2 minutes at 30 Hz. Centrifuge 3 minutes at 1 000 g. Transfer **100 µl** of **supernatant** in a microtube	Transfer **200 µl** of **supernatant** in a microtube.
Lysis	Add **200 µl** of **AL buffer, 20 µl** of **pK** and **100 µl** of **PBS 1X.**		Add **200 µl** of **AL buffer** and **20 µl** of **pK**.
	Vortex and incubate 10 minutes at +56°C.		
Binding preparation	Add **200 µl** of **ethanol 100%**. Homogenise the mixture by pipeting or by vortex.		
Transfer to columns and binding to the membrane	Identify columns, apply the **whole** obtained solution to the corresponding column. Centrifuge 1 minute at 10 000 g. *If the whole sample has not been loaded once, apply the residual volume onto the column and centrifuge 1 minute at 10 000 g.*		
1st wash	Change the collection tube and add **500 µl** of **AW1 buffer** to the column. Centrifuge 1 minute at 10 000 g.		
2nd wash	Change the collection tube and add **500 µl** of **AW2 buffer** to the column. Centrifuge 1 minute at 10 000 g.		
Column dry step	Change the collection tube. Centrifuge 3 minutes at 10 000 g.		
Elution	Transfer the column to a microtube. Add **200 µl** of **AE buffer**. Incubate ~1 minute at room temperature and centrifuge 1 minute at 10 000 g.		
Storage	Close the tubes, identify and store at +2/8°C for 24 hours, then at <-15°C.		

2. Extraction using NucleoSpin® RNA Virus kit

It's a same protocol for the detection of the Classical Swine Fever Virus with ADIAVET™ CSFV REAL TIME (Ref. ADI223).

<table>
<tr><th></th><th>Blood, serum, cell culture supernatant</th><th>Tissue</th><th>Swab</th></tr>
<tr><td rowspan="2">Preparation of the sample</td><td>100 µl (individual or pool)</td><td>20-30 mg (individual or pool)
+ 300 µl of PBS 1X.</td><td>Add 1 ml of PBS 1X.
Vortex.</td></tr>
<tr><td></td><td>Add 1 metal bead in the microtube.
Disrupt 2 minutes at 30 Hz.
Centrifuge 3 minutes at 1 000 g.
Transfer 100 µl of supernatant in a microtube.</td><td>Transfer 100 µl of supernatant in a microtube.</td></tr>
<tr><td rowspan="2">Lysis</td><td colspan="3">Add 400 µl of pre-warmed RAV1 buffer + RNA carrier</td></tr>
<tr><td colspan="3">Vortex and incubate 10 minutes at +70°C.</td></tr>
<tr><td>Binding preparation</td><td colspan="3">Add 400 µl of ethanol 100%.
Homogenise the mixture by pipeting or by vortexing.</td></tr>
<tr><td>Transfer to columns and binding to the membrane</td><td colspan="3">Identify columns, apply 630 µL of the obtained solution to the corresponding column.
Centrifuge 1 minute at 10 000 g.
Apply the residual volume onto the column and centrifuge 1 minute at 10 000 g.</td></tr>
<tr><td>1st wash</td><td colspan="3">Change the collection tube and add 500 µl of RAW buffer to the column.
Centrifuge 1 minute at 10 000 g.</td></tr>
<tr><td>2nd wash</td><td colspan="3">Change the collection tube and add 500 µl of RAV3 to the column.
Centrifuge 1 minute at 10 000 g.</td></tr>
<tr><td>Column dry step</td><td colspan="3">Change the collection tube.
Centrifuge 3 minutes at 10 000 g.</td></tr>
<tr><td>Elution</td><td colspan="3">Transfer the column to a microtube. Add 100 µl of Nuclease-free water.
Incubate ~1 minute at room temperature and centrifuge 1 minute at 10 000 g.</td></tr>
<tr><td>Storage</td><td colspan="3">Close the tubes, identify and store at +2/8°C for 24 hours, then at <-15°C.</td></tr>
</table>

3. Extraction using QIAamp® Viral RNA.

It's a same protocol for the detection of the Classical Swine Fever Virus with ADIAVET™ CSFV REAL TIME (Ref. ADI223).

	Blood, serum, cell culture supernatant	Tissue
Preparation of the sample	**100 µl** (individual or pool)	**20-30 mg** (individual or pool)
Lysis	Add **560 µl AVL+ carrier** Vortex and incubate 10 minutes at room temperature	Add **560 µl AVL+ carrier** Add **1 metal bead** in the microtube. Disrupt 2 minutes at 30 Hz. Centrifuge 2 minutes at 6000 g. Transfer **100 µl** of **supernatant** in a microtube
Binding preparation	Add **560 µl** of **ethanol 100%**. Homogenise the mixture by pipeting or by vortex	
Transfer to columns and binding to the membrane	Identify columns, apply 630 µL of the obtained solution to the corresponding column. Centrifuge 1 minute at 10 000 g. *Apply the residual volume onto the column and centrifuge 1 minute at 10 000 g.*	
1st wash	Change the collection tube and add **500 µl** of **AW1 buffer** to the column. Centrifuge 1 minute at 10 000 g.	
2nd wash	Change the collection tube and add **500 µl** of **AW2 buffer** to the column. Centrifuge 1 minute at 10 000 g.	
Column dry step	Change the collection tube. Centrifuge 3 minutes at 10 000 g.	
Elution	Transfer the column to a microtube. Add **60 µl** of **AVE buffer**. Incubate ~1 minute at room temperature and centrifuge 1 minute at 10 000 g.	
Storage	Close the tubes, identify and store at +2/8°C for 24 hours, then at <-15°C.	

4. Extraction using DNA/RNA magnetic beads kit

It's a same protocol for the detection of the Classical Swine Fever Virus with ADIAVET™ CSFV REAL TIME (Ref. ADI223).

See the NEKF user manual available on the web site mentioned on the certificate of analysis included in the used ADIAVET™ kit.

V. Amplification

a - Determine the number of analysed samples including the controls (e.g. positive and negative extraction controls, positive control of amplification (ASFV CTL+) and No Template Control (NTC)).

b - Defrost the A5 solution at room temperature. Vortex. Dispense **20 µl** of the A5 solution in each PCR tubes or PCR plate wells with a micropipette with a Nuclease-free tip.

c- **Immediately replace the A5 tube at <-15°C and in darkness.**

d- For each sample, the extraction negative control (obligatory) and the extraction positive control (recommended) add **5 µl** of purified extract to the 20 µl of A5 solution.
For the CTL+, add **5 µl** of the solution obtained in § II-3 to the 20 µl of A5 solution.
For the No Template Control (NTC), nothing is added to the A5 solution.
Immediately replace purified DNA extracts at +2/8°C or at **<-15°C**. Take care to have no bubbles in the bottom of the wells.

e- Once all the tubes have been prepared, run the real-time PCR amplification.

The ASFV target is read in FAM. The Internal Control is read in VIC or HEX. The Quencher is non-fluorescent. The solution contains a passive reference ROX for the ABI machines. Fluorescence is read during the elongation step.

The following programs are defined for **ABI Prism** thermocyclers (like 7500, StepOne…) from **Applied Biosystems** (check the "emulation 9600" option if available), for the **MX3005P** and **ARIAMX** of **Agilent** and for **CFX96** of **BioRad**.

<table>
<tr><th colspan="2">Short program of
ADIAVET™ CSFV REAL TIME</th><th colspan="2">Fast Program</th></tr>
<tr><td colspan="2">10 min. 45°C</td><td colspan="2" rowspan="2">2 min. 95°C</td></tr>
<tr><td colspan="2">10 min. 95°C</td></tr>
<tr><td>5 sec 95°C</td><td rowspan="2">45 cycles</td><td>5 sec 95°C</td><td rowspan="2">45 cycles</td></tr>
<tr><td>30 sec. 60°C*</td><td>30 sec 60°C *</td></tr>
</table>

* Note 32 secondes for the ABI7500 **Applied Biosystems**

Contact us if you wish to use other thermalcyclers.

VI. Interpretation of results

1. Definitions

The **« base line »** corresponds to the background of fluorescence and qualifies the non-characteristic part of the curve observed during the first cycles.

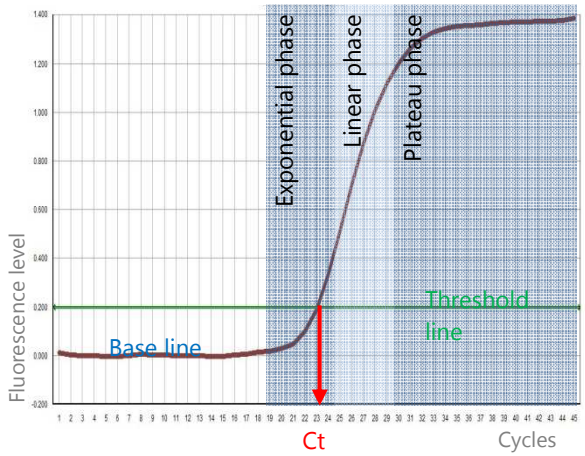
The **« Characteristic amplification curve »** qualifies a fluorescence curve with an exponential phase, a linear phase and a plateau phase.

The **« threshold line »** has to be placed over the background in the exponential phase of a characteristic amplification curve or a group of characteristic amplification curves.

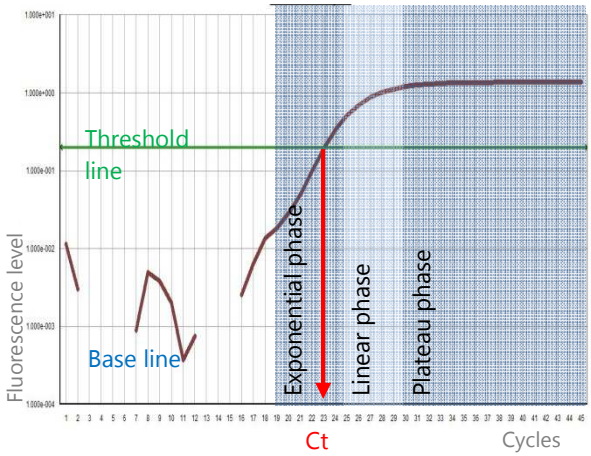
The **« threshold cycle » (Ct)** of a well corresponds, for each detected fluorophore, at the crossing point of the threshold line with the fluorescent curve. The Ct value expressed by the machine for each well depends on the threshold position and on the quantity of target sequences initially present in the PCR well.

Example of characteristic amplification curve

Arithmetic scale of Y axis



Logarithmic scale of Y axis



2. Validation and interpretation of results

Display the FAM curves from the plate and set the threshold value as indicated above. Proceed in the same mean for the VIC/HEX curves.

A. Validation of the run

Amplification is **valid** if the following results are obtained for the controls:

Controls	No Template Control (NTC)	Amplification positive control (CTL+)	Extraction negative control	Extraction positive control *
FAM amplification	No	Yes	No	Yes
VIC/HEX amplification	No	Yes	No	Yes
Validation of	Absence of contamination for amplification	Amplification of the target and internal control	Absence of contamination for extraction	Extraction and amplification steps vérified

* Optional

The indicative Ct values (FAM and VIC/HEX dyes) of the positive control (CTL+) are indicated in the certificate of analysis of the kit.

B. Result interpretation

DNA extraction and amplification for each sample are **valid** if at least a characteristic amplification curve is observed for ASFV (FAM) or for the internal control (VIC/HEX).

Example	A	B	C
FAM amplification	No	Yes	No
VIC/HEX amplification	Yes	No/Yes	No
Result	negative	Positive ASFV	undetermined

The sample is considered as **negative** if a characteristic amplification curve is observed in VIC or HEX without any amplification in FAM (example A).

The sample is considered as **positive** if a characteristic amplification curve is observed in FAM. Internal control can be co-amplified (example B).

A total absence of characteristic amplification curve for a sample (example D) shows a defective DNA extraction (lost or destruction of DNA) or a deficient real-time PCR (inhibitors in the sample, program error or no template added). In this case, we recommend first to repeat the test with pure and tenfold diluted DNA in Nuclease-free water. Then, if the test is still not valid, a new extraction is recommended.

VII. Index of symbols

Symbol	Meaning
	Catalogue number
	Manufacturer
	Upper temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Keep away from sunlight
	For veterinary in vitro use only

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