ADIAVET™ PRRSV REAL TIME

TEST FOR THE DETECTION OF THE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (EUropean and North American) BY REAL-TIME ENZYMATIC AMPLIFICATION (RT-PCR TEST)

References:
ADI133-50 (50 reactions)
ADI133-100 (100 reactions)

VET

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English version
NE133-06
2018/09

ADIAVET™ PRRSV REAL TIME

I. Revision history

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 history.

Release Date	Part Number	Change type	Change summary
2014/12	NE133-04	Correction	The supplier changed NucleoSpin® RNA II designation for NucleoSpin® RNA, in page 6, § III-4.
2014/12	NE133-04	Correction	The supplier changed RA2 designation for RAW2, in page 15, § V-4.
2014/12	NE133-04	Technical change	Addition of "Index of symbols" section, in page 20.
2014/12	NE133-04	Technical change	Removal of reference ADI133-50 (50 reactions)
2016/07	NE133-05	Administrative	Changing logos
2016/07	NE133-05	Administrative	Biosearch legal mention
2016/07	NE133-05	Administrative	Addition of table "Analysis options according to the specimen" §I.3.
2018/09	NE133-06	Administrative	Addition of ADI133-50 reference

II. General information

1. Purpose of the test

ADIAVET™ PRRSV REAL TIME kit is intended to detect the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) type 1 and type 2 using real-time Polymerase Chain Reaction (PCR) technology from tissue swab, from tissue, oral fluid, whole blood, serum and semen specimens of pig.

2. Pathogen

The porcine reproductive and respiratory syndrome is due to a small ribonucleic acid virus, member of the *Arteviridae* family.
This virus multiplies in the alveolar macrophages of lungs, in tonsil macrophages or dendritic cells, in lymphatic nodes, in thymus, spleen, liver, kidneys, surrenals and heart.
Two symptoms are mainly observed: reproduction troubles (late abortions, abnormal lengthening of the gestation period, high neonatal mortality, lower fertility) and respiratory troubles associated to loss of weight and hyperthermia. The virus generally stays several years in a herd.
The problem of the clinical diagnosis is that no lesion or no sign is specific to PRRSV. All the signs are characteristic of PRRSV infection but not differentiable from other diseases. Only the laboratory can confirm or infirm the suspicion.
The detection methods currently used are viral isolation (fastidious method), serology or RT-PCR. Up until now, the virus still remains difficult to control despite the availability of several vaccines. The difficulty is linked, in part, to the genomic and antigenic differences of the PRRSV.
PRRSV was identified simultaneously in Europe and the North America. Since then, genomic and antigenic differences have lead to two distinct genotypes being defined: the European genotype (EU or type 1) and the North American genotype (NA or type 2). Since then, both genotypes spread geographically, radiated genetically and acquired new genotypic and phenotypic characteristics. The evolution of the EU genotype leads to differentiate the PRRSV in three subtypes in which the subtype I is divided in clade. Similar evolution is observed for PRRSV of type 2 that are divided in ten lineages.
Efficient PRRSV control needs to take in consideration the genomic and antigenic differences of the virus. Firstly the virus should be detected. Then, the genetic characteristics of the strain can be determined, for vaccine campaign or epidemiology monitoring for example. The sub-typing strategy is based on the analysis of ORF5 and/or ORF7 genes polymorphism mainly by sequencing.

3. Description and purpose of the test

This test is based first on the reverse transcription (RT) of RNA into complementary DNA. Then, cDNA is amplified (PCR) by a DNA polymerase using specific primers. Both enzymatic reactions occur in the same tube (One-step RT-PCR).
Amplified products are detected in real-time thanks to a specific labelled hydrolysis probe (5'-exonulease technology).

The ADIAVET™ PRRSV REAL TIME kit enables the simultaneous detection of:

- PRRSV type 1 and 2 (probe labelled in FAM),
- RNaseP, an internal control of extraction and amplification steps specific from an endogenous RNA (probe labelled with a fluorochrome read in the same spectra as VIC and HEX).

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

Analysis options according to the specimen:

Specimen	Individual analysis	Pool of sample is possible*, up to
Tissue swab (lung)	☑	3
Tissue (lung)	☑	3
Oral fluid	☑	☒
Whole blood	☑	3
Serum	☑	3
Semen	☑	☒

***** It depends on the epidemiological case and on the quality of the specimen.

III. Material and reagents

1. Reagents provided with the kit

Designation	Reagent	ADI133-50	ADI133-100
A5	Amplification solution	1 x 1000 µl green tube	2 x 1000 µl green tubes
PRRSV CTL+	Positive control PRRSV type 1 and 2	1 purple tube	1 purple tube

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.**
Realtime 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 PRRSV CTL+

Add **200 µl** of Nuclease-free water to the PRRSV CTL+ tube included in the kit. Homogenize tube contents using a mixer such as vortex, at least 20 seconds. Aliquot this solution by 6 or 12 µl and store them to **<-15°C**.
For each analysis, we recommend to use 5 µl of PRRSV CTL+ in a well.

4. Equipment required but not supplied

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 and tubes of 10 or 15 ml
- Universal laboratory mixer mill
- Incubators, heating baths or block heaters
- Instrument for homogenous mixing of tubes
- 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
- Sterile tube of 15 ml
- Powder-free Latex or Nitrile gloves
- Metal beads (tungsten or stainless) 3 mm
- Scalpel blades
- 96-100% ethanol solution
- Sterile demineralised water
- Sterile saline water (NaCl 8.5 g/l)
- PBS buffer
- ß-mercaptoethanol 14.5 M

- Equipment required according to the extraction protocol

Extraction kit's references		Additionnal references	Tissue	Swab	Blood	Serum	Semen	Oral fluids
			Higher number of samples in pool					
			3	3	3	3	0	0
RNeasy®	Qiagen, 50 extractions : ref. 74104 or 250 extractions : ref. 74106	- Buffer EL : Qiagen : ref. 79217 (for bloods with anti-coagulant)	+	+	+			
QIAamp® Viral RNA	Qiagen, 50 extractions : ref. 52904 or 250 extractions : ref. 52906		+	+		+	+	+
NucleoSpin® RNA	Macherey-Nagel, 50 extractions : ref. 740955.50 or 250 extractions : ref. 740955.250		+	+				
NucleoSpin® RNA Virus	Macherey-Nagel, 50 extractions : ref. 740956.50 or 250 extractions : réf. 740956.250		+	+		+		+
Magnetic beads RNA/DNA	Bio-X Diagnotics See user manual NEKF		+	+		+		+

IV. Recommendation before the analysis of samples

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

1. Precautions

ADIAGENE has elaborated this PCR test with the use of extraction kits from Qiagen and Macherey-Nagel. 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 twice 25 minutes at +120°C or once 60 minutes at +121°C. Take a new fraction for each new manipulation to avoid contamination.

2. Storage of samples and RNA extracts

Samples can be stored a couple of days at +2/8°C. After 2 days, we recommend to store them at **<-15°C**.
Samples of blood with anticoagulant reagent must not be frozen.
Extracted RNAs are quite sensitive molecules. Extraction is made at room temperature and should be performed as fast as possible to avoid degradations. We then recommend to read the entire protocol before starting the test and to rigorously respect it. Crude extracts should be stored at the end of extraction on melting ice or at +2/8°C for few hours, then at **<-15°C**.

3. Samples preparation

See § IV for the extraction and purification of RNA.

4. Controls to include

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 kit.

- The internal endogenous control (RNaseP) naturally found in the samples allows verifying the extraction and amplification steps of each sample.
- The PRRSV CTL+ allows validating the amplification of the target.

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 (e.g. the normative requirement and recommendation for the development and the validation of veterinary PCR NF U47-600 suggests the use of 1 negative control for 24 samples or 4 negative samples for a 96 wells-plate). This control could be a negative matrix, or a buffer used for dilutions.

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

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

V. Extraction and purification

1. Using QIAamp® Viral RNA kit

A. Sample preparation

a) From tissue

Grind* **20 mg** of **tissue** or n x 20 mg of a pool of n tissues (3 maximum) in **560 µl** of **AVL buffer + RNA carrier**.
Briefly centrifuge.
Continue following the table below.

* For example, using a Mixer Mill: add 1 metal bead (3 mm), grind 2 minutes at 30 Hz.

b) From swab

Add **2 ml** of **sterile saline water** in the tube of the swab, homogenize.
NB: possibility to pool until 3 swabs. Transfer the supernatant obtained in the next tube of swab.
Press each swab to collect as many liquid as possible.
Transfer the liquid in a 2 ml-microtube.
Place **140 µl** of **sample** in a microtube.
Continue following the table below.

c) From serum

Place **140 µl** of **serum** or pool of sera (3 maximum) in a microtube.
Continue following the table below.

d) From semen

Place **1 ml** of **semen** (no pool allowed) in a microtube.
Centrifuge 2 minutes at 3000 g at +4°C.
Place **140 µl** of **supernatant** in a microtube.
Continue following the table below.

e) From oral fluids

Place **140 µl** of **oral fluids** in a microtube.
NB: optional *– An EPC-extraction can be added; contact us for further details*
Continue following the table below.

B. RNA extraction and purification

All the centrifugations are performed at room temperature, except if specified.

	Tissue	**Swab** **Serum** **Semen** **Oral fluids**
Lysis	From sample prepared as described before.	From **140 µl** of **sample** prepared as described before.
		Add **560 µl** of **AVL buffer + RNA carrier**. Homogenize ~15 seconds. Incubate at room temperature during 10 minutes.
Binding preparation	Add **560 µl** of **ethanol 100%**. Homogenize by pipetting (~10 times) or by using a mixer such as vortex (~15 seconds).	
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. Change the collection tube, put the rest of the mix on the column and centrifuge 1 minute at 10 000 g.	
1st wash	Change the collection tube and add **500 µl** of **AW1 buffer**. Centrifuge 1 minute at 10 000 g.	
2nd wash	Change the collection tube and add **500 µl** of **AW2 buffer**. Centrifuge 1 minutes 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 on ice if using immediately, or at **<-15°C**.	

2. Using RNeasy® kit

A. Sample preparation

a) From tissue

Grind* **0.1g** of **tissue** or n x 0.1g of a pool of n tissues (3 maximum) in **1 ml** of **sterile saline water**.
Centrifuge 2 minutes at 830 g.
Place **140 µl** of the **supernatant** in a microtube.
Continue following the table below.

* For example, using a Mixer Mill: add 1 metal bead (3 mm), grind 2 minutes at 30 Hz.

b) From swab

Place a **swab** or a pool of swabs (3 maximum) in a 2 ml-microtube.
Continue following the table below.

c) From blood

Place **500 µl** of **blood** or 500 µl of a pool of bloods (3 maximum) in a 15 ml-tube.
Add 2.5 ml of EL buffer, homogenize.
Incubate on ice for 15 minutes (homogenize twice during this lysis step).
Centrifuge 10 minutes at 1000 g at +4°C.
Discard the supernatant.
Add **2 ml** of **EL buffer** to the pellet and homogenize.
Centrifuge 10 minutes at 1000 g at +4°C.
Discard the supernatant.*
Continue following the table below.

* The pellet can then be stored at – 70 +/-10°C during several months.

B. RNA extraction and purification

All the centrifugations are performed at room temperature, except if specified.

	Blood	Swab	Tissue
Lysis	Add **300 µl** of **RLT buffer** + **β–mercaptoethanol** at 10 µl/ml to the pellet.	Add **300 µl** of **RLT buffer** + **β-mercaptoethanol** at 10 µl/ml. Mix. Press each swab to collect as many liquid as possible and discrad the swab.	Add **300 µl** of **RLT buffer** + **β-mercaptoethanol** at 10 µl/ml to the **supernatant**. Incubate 15 minutes at room temperature and homogenize now and then.
Binding preparation	Add **300 µl** of **ethanol 70%**. Homogenize by pipetting (~10 times) or by using a mixer such as vortex (~15 seconds).		
Transfer to columns and binding to the membrane	Identify columns, transfer the **whole content** to the corresponding column. Centrifuge 1 minute at 10 000 g.		
1st wash	Change the collection tube and add **700 µl** of **RW1 buffer**. Centrifuge 1 minute at 10 000 g.		
2nd wash	Change the collection tube and add **500 µl** of **RPE buffer**. 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 **Nuclease-free water**. Incubate ~1 minute at room temperature and centrifuge 1 minute at 10 000 g.		
Storage	Close the tubes, identify and store on ice if using immediately or at <-15°C.		

3. Using Nucleospin® RNA Virus kit

A. Sample preparation

a) From tissue

Grind* **20 mg** of **tissue** or n x 20 mg of a pool of n tissues (3 maximum) in **560 µl** of **RAV1 buffer + carrier** (pre-warmed at 56°C).
Briefly centrifuge.
Continue following the table below.

* For example, using a Mixer Mill: add 1 metal bead (3 mm), grind 2 minutes at 30 Hz.

b) From swab

Add **2 ml** of **sterile saline water** in the tube of the swab, homogenize.
NB: possibility to pool until 3 swabs. Transfer the supernatant obtained in the next tube of swab.
Press each swab to collect as many liquid as possible.
Transfer the liquid in a 2 ml-microtube.
Place **140 µl** of **sample** in a microtube.
Continue following the table below.

c) From serum

Place **140 µl** of **serum** or pool of sera (3 maximum) in a microtube.
Continue following the table below.

d) From oral fluids

Place **140 µl** of **oral fluids** in a microtube.
NB: optional *– An EPC-extraction can be added; contact us for further details*
Continue following the table below.

B. RNA extraction and purification

All the centrifugations are performed at room temperature, except if specified.

	Tissue	**Swab** **Serum** **Oral fluids**
Lysis	From sample prepared as described before.	From **140 µl** of **sample** prepared as described before.
		Add **560 µl** of **RAV1 buffer + carrier** pre-warmed at +56°C. Homogenize ~15 seconds. Incubate at room temperature during 10 minutes.
Binding preparation	Add **560 µl** of **ethanol 100%**. Homogenize by pipetting (~10 times) or by using a mixer such as vortex (~15 seconds).	
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. Change the collection tube, put the rest of the mix on the column and centrifuge 1 minute at 10 000 g.	
1st wash	Change the collection tube and add **500 µl** of **RAW buffer**. Centrifuge 1 minute at 10 000 g.	
2nd wash	Change the collection tube and add **500 µl** of **RAV3 buffer**. Centrifuge 1 minutes 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 **Nuclease-free water**. Incubate ~1 minute at room temperature and centrifuge 1 minute at 10 000 g.	
Storage	Close the tubes, identify and store on ice if using immediately or at <-15°C.	

4. Using Nucleospin® RNA kit

All the centrifugations are performed at room temperature, except if specified.

	Swab	**Tissue**
Lysis	Place a **swab** or a pool of swabs (3 maximum) in a 2 ml-microtube. Add **300 µl** of **RA1 buffer** + **β-mercaptoethanol** at 10 µl/ml. Mix. Press each swab to collect as many liquid as possible and discrad the swab.	Grind* **0.1g** of **tissue** or **n x 0.1g** of a pool of n tissues (3 maximum) in 1 ml of sterile saline water. Centrifuge 2 minutes at 830 g. Place 140 µl of the supernatant in a microtube. Add **300 µl** of **RA1 buffer** + **β-mercaptoethanol** at 10 µl/ml to the **supernatant**. Incubate 15 minutes at room temperature and homogenize now and then.
Binding preparation	Add **300 µl** of **ethanol 70%**. Homogenize by pipetting (~10 times) or by using a mixer such as vortex (~15 seconds).	
Transfer to columns and binding to the membrane	Identify columns, transfer the **whole content** to the corresponding column. Centrifuge 1 minute at 11 000 g.	
1st wash	Change the collection tube and add **350 µl** of **MDB buffer**. Centrifuge 1 minute at 11 000 g.	
2nd wash	Change the collection tube and add **200 µl** of **RAW2 buffer**. Centrifuge 1 minute at 11 000 g.	
3rd wash	Change the collection tube and add **600 µl** of **RA3 buffer**. Centrifuge 1 minute at 11 000 g.	
4th wash	Change the collection tube and add **250 µl** of **RA3 buffer**. Centrifuge 5 minutes at 11 000 g.	
Elution	Transfer the column to a microtube. Add **60 µl** of **Nuclease-free water**. Incubate ~1 minute at room temperature and centrifuge 1 minute at 10 000 g.	
Storage	Close the tubes, identify and store on ice if using immediately or at <-15°C.	

* For example, using a Mixer Mill: add 1 metal bead (3 mm), grind 2 minutes at 30 Hz.

5. Using DNA/RNA magnetic beads kit

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

VI. Amplification

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

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

c- **Immediately replace the A5 solution 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 PCR reagent control (NTC), nothing is added to the A5 solution.
Immediately replace purified RNA extracts on melting ice or at <-15°C. Take care to have no bubbles in the bottom of the wells.

e- Store the plate or the tubes on melting ice or at +2/8°C until the cycler is programmed and start quickly the run after you have placed the plate or the tubes in the cycler.

The PRRSV 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 (1 minute at 60°C).

The following program is defined for **ABI Prism** thermalcyclers (like 7500, StepOne...) from **Applied Biosystems** (check the "emulation 9600" option if available), for the **Rotorgene** from **Qiagen** and for the **Chromo 4** from **Biorad**:
10 minutes 45°C
10 minutes 95°C
15 seconds at 95°C and 1 minute at 60°C during 45 cycles

This program is concerning the **MX3000P** and **MX3005P** of **Stratagene**:
10 minutes 45°C
10 minutes 95°C
30 seconds at 95°C and 1 minute at 60°C during 45 cycles

Roche diagnostic: LightCycler 2*, LightCycler 480*
**** NOTE:*** *The use of LightCycler thermalcyclers requires a calibration manipulation. Adiagene will furnish process chart and reagents required for this calibration.*

Contact us if you wish to use other thermalcyclers.

VII. 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.

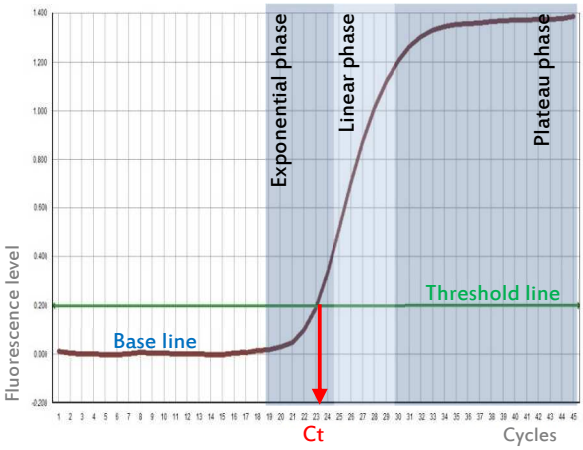
The **« Characteristic amplification curve »** qualifies a fluorescence curve with an exponential phase, a linear phase and a plateau phase.

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.

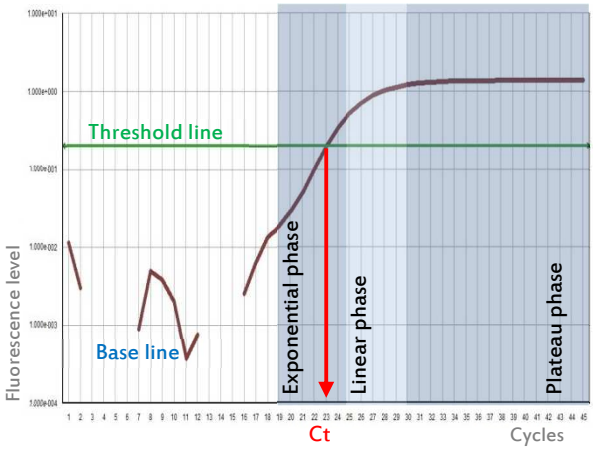
The **« threshold cycle » (Ct)** of a well corresponds, for each detected fluorophore, at the crossing point of the threshold line with the fluorescent curve. The Ct value expressed by the machine for each well depends on the threshold position and on the quantity of target sequences initially present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

A. Validation of the run

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

Controls	Reagent control (NTC)	PRRSV CTL+	Extraction negative control	Extraction positive control *
FAM amplification	no	yes	no	yes
VIC/HEX amplification	no	no/yes	no/yes	no/yes
Validation of	Absence of contamination for amplification	Amplification of the target	Absence of contamination for extraction	Extraction and amplification steps

* Optional

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

B. Result interpretation

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

Example	A	B	C	D
FAM amplification	no	yes	yes	no
VIC/HEX amplification	yes	no	yes	no
Result	negative	positive	positive	undetermined

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

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

A total absence of characteristic amplification curve for a sample (example D) shows a defective RNA extraction (lost or destruction of RNA) 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 RNA in Nuclease-free water. Then, if the test is still not valid, a new extraction is recommended.

VIII. 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 – For animal use only

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