ADIAVET™ EU/NA PRRSV REAL TIME

TEST FOR THE DETECTION AND QUANTIFICATION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS BY REAL TIME ENZYMATIC DNA AMPLIFICATION (RT-PCR TEST)

Reference:
ADI134-100 (100 reactions)

VET

English version
NE134-01
2021/10

ADIAVET™ EU/NA PRRSV REAL TIME

I. Revision history

N/A	Not Applicable (first publication)
Correction	Document anomalies correction
Technical change	Addition, modification and/or removal of information related to the product
Administrative	Non technical modification noticeable by the user

Note: Minor typographical, grammatical or changes related to formatting are not included in the revision history.

Release Date	Part Number	Change type	Change summary
2021/10	NE134-01	NA	First publication

II. General information

1. Test purpose

ADIAVET™ EU/NA PRRSV REAL TIME allows detection and differentiate the European (EU) and the North American (NA) genotypes of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) using real-time Polymerase Chain Reaction (PCR) from serum, blood, tissue, bronchoalveolar lavages (BALs) and oral fluid. This kit also allows quantification of the viral load in a given sample.

2. Pathogen

The Porcine Reproductive and Respiratory Syndrome is caused by a small ribonucleic acid virus belonging to the *Arteviridea* family. PRRS is one of the major porcine diseases and leads to important economic loss worldwide. The virus can be divided in 2 groups based on genetic differences: PRRSV I (so called European strains or EU) and PRRSV II (so called North American strains or NA) (Adam *et al.*, 2016; Kuhn *et al.*, 2016).
Two main symptoms can be observed after infection: reproductive issues (late abortion, abnormaly long pregnancy, high rate of piglets' death, fertility decrease) and respiratory issues associated with hyperthermia and loss of appetite.
PRRS can be found in Europe and North America, but has also been identified in China since 1995. It's also present in Japan, Vietnam, the Philippines, Malaysia and Korea among other countries in Asia. Australie, New-Zealand, India, some european countries and some parts of Africa are as of today free of the disease (OIE website).
PRRSV I/EU and PRRSV II/NA lead to similar early clinical symptoms after infection but with very different virulence (Done *et al.*, 1996). Commercial vaccines exist but only allow mild cross-protection against different infectious genotypes (Han *et al.*, 2014; Jeong *et al.*, 2018).
Recent studies have shown that not all PRRSV I/EU strains are genetically close and that a cluster of strains in eastern Europe (starting from the western border or Poland) can be divided in 4 sub-types, while a single sub-type exist in western and central europe (Balka *et al.*, 2018).
Since different PRRSV genotypes can be found in a herd, the fight against the virus is difficult. A fast, efficient and easy detection of different PRRSV genotypes becomes crucial in order to manage infections. Hence, RT-qPCR is the recommended method for PRRSV identification or infections control.

3. Description and purpose of the test

This test is based on the reverse transcription of RNA into complementary DNA (cDNA). The cDNA is then amplified by Polymerase Chain Reaction (PCR) thanks to specific primers and a DNA polymerase. 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™ EU/NA PRRSV REAL TIME enables the simultaneous detection of:

- PRRSV EU genotype (probe labeled in FAM).
- PRRSV NA genotype (probe labeled in Cy5).
- RNase P gene, an internal control of extraction and amplification specific fromendogenous DNA (probe labeled with a fluorochrome read in the same spectra as VIC and HEX).

The « EU PRRSV CTL+ » et « NA PRRSV CTL+ » contain known amounts of DNA allowing quantification of positive samples using a 5-points range built with the positive controls. The unit of measure of the number of copies by PCR.

Bio-X Diagnostics has validated this test using DNA purification kits (Bio-X Diagnostics and Qiagen). 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 possible*, up to**
Tissue (lung, heart…)	☑	3
Whole blood / serum	☑	3
Saliva	☑	3
Oral fluids, bronchoalveolar lavages (BALs)	☑	☒

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

III. Material and reagents

1. Reagents provided with the kit

REF ADI134-100		
A5	amplification solution	2 x 1000 µl tubes with green caps (ready to use)
EU PRRSV CTL+	PRRSV EU positive control	1 tube with stripped purple caps (to be reconstituted)
NA PRRSV CTL+	PRRSV NA positive control	1 tube with stripped purple caps (to be reconstituted)
NF-Water	Nuclease free water	2 x 1000 µl tubes with white cap (ready-to-use)

2. Validity and storage

After reception, the kit must be stored at < **-15°C**.
It is recommended to aliquot the « A5 » solution if it is envisioned it will be defrosted more than 3 times.
Do not defrost reagents more than 3 times.
Realtime reagents are sensitive to light: store them in the dark.
The A5 reagent is ready to use for PCR reaction.
Do not mix reagents from two different batches.

3. Use of controls

These controls must be manipulated with extreme care since they are highly concentrated in both targets and could lead to contaminations.

Add **200 µl** of "NF-Water" to « EU PRRSV CTL+ » or « NA PRRSV CTL+ » included in the kit. Homogenize tube contents using a mixer such as vortex, at least 20 seconds, until complete dissolution of the blue pellet. Aliquot this solution by 6 or 12 µL and store them at < -15°C.

These PRRSV CTL+ should not be defrosted more than 3 times.

Realize a standard range with 10-fold serial dilutions of EU PRRSV CTL+ and NA PRRSV CTL+ using NF-Water (recommended) or Nuclease-free water. Use 5 µl of each point of the range.

Standard range	***Number of PRRSV EU RNA copies / PCR***	***Number of PRRSV NA RNA copies / PCR***
Pure	2.10^6	10^6
1/10	2.10^5	10^5
1/100	2.10^4	10^4
1/1000	2.10^3	10^3
1/10000	2.10^2	10^2

If quantification is not needed, we recommend using 5 µl of the 1/1000 dilutions for each control.

4. Equipment required but not supplied with the kit

Material should be Nuclease-fre (e.g.: autoclaved 25 minuted twice at +120°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, tubes of 5, 10 or 15 ml
- Etuve, heating baths or block heaters (+70°C)
- Instrument for homogenous mixing of tubes (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
- Sterile tubes of 5, 10 or 15 ml
- Powder-free latex gloves
- 96-100 % ethanol solution
- Scalpel blades
- PBS buffer 1X pH 7.4
- Nuclease-free water

- RNA extraction kit (individual columns):

QIAamp® Viral RNA Mini Kit (Qiagen, 50 tests: ref. 52904 or 250 tests: ref. 52906)

Or

- Automated RNA/DNA extraction kit (magnetic beads):

ADIAMAG (Bio-X Diagnostics ; 200 extractions : ref. NADI003) or ADIAMAG XL (Bio-X Diagnostics ; 800 extractions : ref. NADI003-XL).

IV. Samples preparation and controls

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

1. Precautions

Adiagène has validated this PCR test using Bio-X Diagnostics and Qiagen extraction kits. Other extraction kits can be used after validation by the user.

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

Some kits include and/or need toxic reagents. These reagents should be manipulated with gloves and into chemical cabinets.

Only appropriately trained personnel should perform this test. The quality of the obtained results depends on rigorous respect of good laboratory practices. Ensure the accuracy and precision of the micropipettes used.

The PCR generates large amount of DNA. A few molecules of amplified products are sufficient to generate a positive result. It is Hence, it is important to reserve 2 rooms, one for the manipulation of the samples to be tested, and the other one for amplified products analysis. PCR tubes should not be opened 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 aliquots of NF-water and to take a new aliquot for each new manipulation, to avoid contamination.

2. Samples and DNA extracts storage

Samples can be stored for a couple of days at +2/8°C. After 2 days, we recommend storing them at < -15°C. Note: blood samples containing blood thiner should never be frozen and must be stored at +4°C.
Extracted RNA are sensitive molecules. After extraction, performed as fast as possible to avoid degradation, RNA extracts can be stored at +2/8°C for a few hours. For long term storage, they should be kept at < -15°C.

3. Controls to include

Controls allow monitoring of results consistency.
They must be included on each PCR run performed.

Each step of the analytical process (extraction and amplification), no matter the matrix, is validated thanks to the use of controls included in the kit :

- Internal endogenous control (RNaseP) is present in the animal cells and allows validation of extraction and amplification for each sample.
- « EU PRRSV CTL+ » and « NA PRRSV CTL+ » allow validation of the target amplification.

Other control could be included according to the process of laboratory:

- **Extraction negative control :**

To verify the absence of cross-contamination, a negative control can be included per trial. This control is a negative sample, for example the buffer used for dilutions.

- **EU or NA Extraction positive control :**

A positive control, containing EU and/or NA PRRSV, can be added in each trial. This control can be a known positive sample available in the laboratory or a negative sample spiked with a PRRSV solution. It will be close to the limit of detection of the method (1 to 100x LOD_{method}) and will be informative regarding the fidelity of the obtained results between different trials.

V. Extraction and purification

1. Using QIAamp Viral RNA kit

A. Samples preparation

a) From tissues

Grind* **20 mg** or n x 20 mg of a pool of n tissues (3 maximum) in **560 µl** of **AVL** buffer + **Carrier RNA**.
Spin briefly.
Proceed according to the following table.
* 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 and 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 liquids 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 or blood

Transfer **140 µl** of **serum** or **blood** in a microtube.
Proceed according to the following table.

d) From saliva

Transfer **140 µl** of **saliva** in a microtube.
Proceed according to the following table.

e) From oral fluids

Place **140 µl** of sample in a microtube.
Continue following the table below.

f) Bronchoalveolar lavages (BALs)

Transfer **1 ml** of sample in microtube.
Centrifuge 30 minutes at 10 000g.
Discard the supernatant.
Continue following the table below.

B. RNA extraction and purification

All centrifugations are performed at room temperature, except if mentioned otherwise.

<table>
<tr><th></th><th>Tissue</th><th>Swab
Serum/blood
Saliva
Oral fluids/BALs</th></tr>
<tr><td rowspan="2">Lysis</td><td rowspan="2">From samples prepared as described before.</td><td>From sample prepared as described before.</td></tr>
<tr><td>Add 560 µl of AVL buffer + RNA carrier.
Homogenize ~15 seconds.
Incubate at room temperature during 10 minutes.</td></tr>
<tr><td>Binding preparation</td><td colspan="2">Add 560 µl of ethanol 100%.
Homogenize by pipetting (~10 times) or by using a mixer such as vortex (~15 seconds).</td></tr>
<tr><td>Transfer to columns and binding to the membrane</td><td colspan="2">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.</td></tr>
<tr><td>1st wash</td><td colspan="2">Change the collection tube and add 500 µl of AW1 buffer.
Centrifuge 1 minute at 10 000 g.</td></tr>
<tr><td>2nd wash</td><td colspan="2">Change the collection tube and add 500 µl of AW2 buffer.
Centrifuge 1 minutes at 10 000 g.</td></tr>
<tr><td>Column dry step</td><td colspan="2">Change the collection tube.
Centrifuge 3 minutes at 10 000 g.</td></tr>
<tr><td>Elution</td><td colspan="2">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.</td></tr>
<tr><td>Storage</td><td colspan="2">Close the tubes, identify and store on ice if using immediately or at < -15°C.</td></tr>
</table>

2. Using ADIAMAG 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 analyze, including the controls to include.

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 dark.**

d – For each sample, add **5 µl** of purified RNA or control to the 20 µl of A5 solution. For the negative control of amplification (NTC), nothing is added to the A5 solution.

Immediately replace the purified RNA extracts at +2/8°C or at < -15°C. Pay attention to bubbles in the bottom of the wells. In case bubbles are present, spin briefly the tubes or plates to eliminate them.

e- Start the run quickly after introduction of the tubes or plate in the thermalcycler.
PRRSV EU target is read in **FAM** while **PRRSV NA** is read in **Cy5**. The internal control is read is HEX or VIC. The Quencher is non-fluorescent. The A5 solution contains a passive ROX reference for ABI machines. Fluorescence is read during the elongation step (1 minute at 60°C).

The following program has been defined for **ABI Prism** thermalcyclers (type 7500, QS5…) from **Applied Biosystems** (check the « emulation 9600 » option if available), for the **MX3005P** and **AriaMx** from **Agilent** and for the **CFX96** from **Biorad**:

Standard program	
10 min. 45°C	
10 min. 95°C	
15 sec 95°C*	45 cycles
1 min. 60°C	

* Use 30 seconds for the MX3005P
For Applied ABI7500 et QS5 machines, set quenchers on « none ».

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

Contact us if you wish to use other thermalcyclers.

VII. Results interpretation

1. Definitions

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

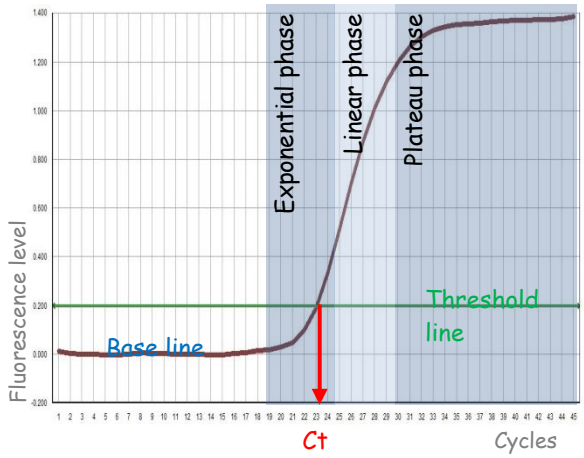
The **« Characteristic amplification curve »** qualifies a fluorescence curve with an exponential phase, a linear phase and a plateau phase.

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.

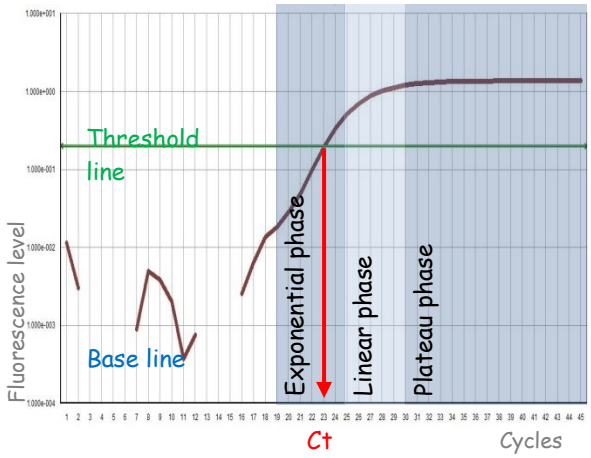
The **« threshold cycle » (Ct)** of a well corresponds, for each detected fluorophore, to the crossing point of the threshold line and fluorescent curve. The Ct value expressed by the machine for each well depends on the threshold position and on the quantity of target sequences initially present.

Example of characteristic amplification curve

Arithmetic scale of Y axis



Logarithmic scale of Y axis



2. Qualitative results validation and interpretation

Display the FAM curves from the plate and set the threshold value as indicated above.
Repeat for the Cy5 and VIC or HEX curves.

A. Run validation

The PCR run is considered valid if the following results are obtained according to the controls included :

Control	Negative control of amplification (NTC)	EU PRRSV CTL+	NA PRRSV CTL+	Extraction negative control	EU positive extraction control	NA positive extraction control
FAM amplification	No	Yes	No	No	Yes	No
Cy5 amplification	No	No	Yes	No	No	Yes
VIC/HEX amplification	No	No	No	No/Yes	No/Yes	No/Yes
Validation of	Absence of contamination during amplification	EU target amplification	NA target amplification	Absence of contamination during extraction	Extraction and amplification steps	Extraction and amplification steps

The expected Ct values in FAM, Cy5 and VIC/HEX for the positive controls (« EU PRRSV CTL+ » and « NA PRRSV CTL+ ») are indicated on the certificate of analysis of the kit.

B. Results interpretation

RNA extraction and amplification for each sample are considered **valid** if at least a characteristic amplification curve is observed for EU PRRSV (FAM), NA PRRSV (Cy5) and/or internal control (VIC ou HEX).

Exemple	A	B	C	D	E
FAM amplification	No	Yes	No	Yes	No
Cy5 amplification	No	No	Yes	Yes	No
VIC/HEX amplification	Yes	Yes/No	Yes/No	Yes/No	No
Result	Undetected	EU PRRSV detected	NA PRRSV detected	EU and NA PRRSV detected	Undetermined To be processed again

The sample is considered as **undetected** if a characteristic amplification curve is observed in VIC or HEX but not in FAM (example A).
The sample is considered as **detected** if a characteristic amplification curve is observed in FAM and/or Cy5 (example B, C and D).

A total absence of characteristic amplification curve for a sample (example E) shows a defective RNA extraction (RNA loss or destruction) or a deficient real-time PCR (inhibitors in the sample, program error or no template added). In this case, we recommend to first repeat the test with pure and ten-fold diluted RNA in Nuclease-free water. If the test remains not valid, a new RNA extraction is recommended.

3. Quantitative results validation and interpretation

Display the FAM curves from the plate and set the threshold value as indicated above.
Repeat for the Cy5 and VIC or HEX curves.

A. Standard range and controls validation

The PCR run is considered valid if the following results are obtained according to the controls included :

Control	***PRRSV quantity (copies / PCR)***	**Amplification in FAM (or Cy5)**	**Amplification in VIC/HEX**
PRRSV EU or NA CTL+ pure	2.10^6 or 10^6	Yes	No
PRRSV EU or NA CTL+ 1/10	2.10^5 or 10^5	Yes	No
PRRSV EU or NA CTL+ 1/100	2.10^4 or 10^4	Yes	No
PRRSV EU or NA CTL+ 1/1000	2.10^3 or 10^3	Yes	No
PRRSV EU or NA CTL+ 1/10000	2.10^2 or 10^2	Yes	No
Negative control of amplification	0	No	No
Positive control of extraction	To be determined	Yes	No/Yes
Negative control of extraction	0	No	No/Yes

The expected Ct values in FAM, Cy5 and VIC/HEX for the positive controls (« EU PRRSV CTL+ » and « NA PRRSV CTL+ ») are indicated on the certificate of analysis of the kit.
If the results of the controls correspond to the table above, samples analysis is allowed.

For the quantitative interpretation, PCR efficiency (Eff.) must be checked. Ct values obtained for successive dilutions should increase proportionally with the dilution (3,33 Ct for a 1/10 dilution). Softwares usually supplied with thermalcyclers can establish a calibration line (cycles number = f (Log concentration)) and determine the standard curve equation.
(y = ax + b) along with the PCR efficiency:
Eff. % = $(10^{(-1/a)} - 1) \times 100$

Before results analysis, the standard curve must be validated:

- The 5 points of the standard range must be amplified. One point of the range can however be removed, assuming it is not one of the two extremes.
- R^2 has to be greater than 0,9.
- Efficiency must be comprised between 75 et 125 %.
- The points must be homogenously spread.

B. Samples results expression

Samples results are represented in the following table:

Example	A	B	C	D	E
FAM or Cy5 amplification	Yes $Ct_{sample} < LOQ_{MAX}$	Yes $LOQ_{MAX} < Ct_{sample} < LOQ_{METHODE}$	Yes $Ct_{sample} > LOQ_{METHODE}$	No	No
VIC/HEX amplification	Yes/No	Yes/No	Yes/No	Yes	No
Result expression	Quantity detected higher than LOQ_{MAX}	Detected and quantifiable*	Quantity detected lower than $LOQ_{METHODE}$	No PRRSV detected	Undetermined: Process again

**in the domain of quantification according to the method used (see kit DV).*
To facilitate the expression and quantitative interpretation of a sample, a computing file can be transmitted upon request.

EU PRRSV and/or NA PRRSV are considered as **detected** in the sampleif a characteristic amplification curve is observed in FAM and/or Cy5. The internal control can be co-amplified in VIC/HEX. Competition can sometimes occur if the sample is very highly loaded in PRRSV target.

When the sample is « quantifiable », the corresponding PRRSV concentration is determined thanks to the equation obtained using the standard range:

$$x = 10^{\left(\frac{y-b}{a}\right)}$$

Where x : PRRSV concentration
y : Ct value in FAM (or Cy5) of the positive sample to be quantified
b : curve intersect
a : curve slope

A multiplying coefficient must be added in order to quantify in copies/gram or copies/ml of sample.
The multiplying coefficient to apply depends on the matrix and extraction method used.
Example of multiplying coefficient after the use of ADIAMAG extraction kit according to the user manual NEKF:

Matrix	Multiplying coefficient according to ADIAMAG extraction	Unit
Serum / Blood	200	copies / ml
Tissue	10 000	copies / g

The sample is considered **undetected** when a characteristic amplification curve is observed in VIC/HEX while no amplification is observed in FAM.

The absence of characteristic amplification curves for a sample indicates a defective RT-PCR (PCR inhibitors in the sample, poor cells amount during extraction, program error…). In this case, we recommend to first repeat the test with pure and ten-fold diluted RNA in Nuclease-free water to dilute potential inhibitors. If the test remains not valid, a new extraction is recommended.

Make sure to accordingly integrate to 1/10 dilution factor during quantification.

VIII. Bibliography

M.J. Adams, E.J. Lefkowitz, A.M. Q. King, B. Harrach, R.L. Harrison, N.J. Knowles, A.M. Kropinski, M. Krupovic, J.H. Kuhn, A.R. Mushegian, M. Nibert, S. Sabanadzovic, H. Sanfaçon, S.G. Siddell, P. Simmonds, A. Varsani, F.M. Zerbini, A.E. Gorbalenya, A.J. Davison (2016). Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2016). Arch Virol. 161:2921-2949.

G. Balka, K. Podgorska, M.S. Brar, A. Balint, D. Cadar, V. Celer, L. Dénes, Z. Dirbakova, A. Jedryczko, L. Marton, D. Novosel, T. Petrovic, I. Sirakov, D. Szalay, I. Toplak, F.C.C. Leung, T. Stadejek (2018). Scientific Reports. 8:7811.

S.H. Done, D.J. Paton, M.E. White (1996). Porcine reproductive and respiratory syndrome (PRRS): a review, with emphasis on pathological, virological and diagnostic aspects. Br Vet L. 152(2):153-174.

K. Han, H.W. Seo, C. Park, C Chae (2014). Vaccination of sows against type 2 Porcine Reproductive and Respiratory Syndrom Virus (PRRSV) before artificial insemination protects against type 2 PRRSV challenge but does not protect against type 1 PRRSV challenge in late gestation. Vet Research. 45:12.

J. Jeong, C. Park, T. Oh, K.H. Park, S. Yang, I. Kang, S.J. Park, C. Chae (2018). Cross-protection of a modified-live porcine reproductive and respiratory syndrome virus (PRRSV)-2 vaccine against a heterologous PRRSV-1 challenge in late-term pregnancy. Vet Microbiology. 223:119-125.

J.H. Kuhn, M. Lauck, A.L. Bailey, A.M. Shchetinin, T.V. Vishnevskaya, Y. Bào, T. Fei Fan Ng, M. LeBreton, B.S. Schneider, A. Gillis, U. Tamoufe, J. Le Doux Diffo, J.M. Takuo, N.O. Kondov, L.L. Coffey, N.D. Wolfe, E. Delwart, A.N. Clawson, E. Postnikova, L. Bollinger, M.G. Lackemeyer, S.R. Radoshitzky, G. Palacios, J. Wada, Z.V. Shevtsova, P.B. Jahrling, B.A. Lapin, P.G. Deriabin, M. Dunowska, S.V. Alkhovsky, J. Rogers, T.C. Friedrich, D.H. O'Connor, T.L. Goldberg (2016). Reorganization and expansion of the nidoviral family Arteriviridae. Arch Virol. 161:755-768.

IX. Symbols index

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

Bio-X Diagnostics
9, rue Gabriel Calloët-Kerbrat
22440 Ploufragan - France

RCS 417 876 299
Tel. +33 (0)2 96 68 40 20
www.biox.com