Instruction for Use

# respiPig 1

## real time RT-PCR Kit

For the qualitative *in-vitro* detection of RNA from Porcine Reproductive and Respiratory Syndrome Viruses, Influenza Viruses Type A, and the DNA of Mycoplasma hyopneumoniae.

| | | |
|---|---|---|
| REF | G01105-32 | G01105-96 |
| Σ | 32 | 96 |
| - 18°C | | |

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# 1 Intended Use

The respiPig 1 real time RT-PCR Kit is an assay for the simultaneous detection of the RNA of the North American (NA), European (EU) genotypes of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), the RNA of type A influenza viruses (Flu A) and the DNA of Mycoplasma hyopneumoniae (M. hyopneumoniae). Extracted RNA and DNA from swine blood, serum, tissue, bronchial swabs, saliva, bronchial lavage, semen, and cell culture supernatant samples can be used for analysis.

# 2 Pathogen Information

**PRRSV** infections are highly prevalent in swine and lead to high economical losses. The panzootic disease causes reproductive failure in breeding stock and respiratory tract illness in young pigs. PRRSV is a member of the genus *Arterivirus*, family Arteriviridae, order Nidovirales. The two prototype strains of PRRSV are the North American strain, VR-2332, and the European strain, the Lelystad virus (LV). The European and North American PRRSV strains cause similar clinical symptoms, but represent two distinct viral genotypes whose genomes diverge by approximately 40 %.

**Swine influenza** is a respiratory disease of pigs caused by type A influenza viruses that regularly cause outbreaks of influenza in pigs. Like human influenza viruses, there are different subtypes and strains of swine influenza viruses. The main swine influenza viruses circulating in U.S. pigs in recent years have been, swine triple reassortant (tr) H1N1 influenzavirus, trH3N2 virus, and trH1N2. The incubation period is short, less than 48 hours. The onset can be extremely rapid and dramatic. The classical picture is a house full of pigs that are normal on one day and most of them are prostrate and breathing heavily by the following morning. Severe coughing and laboured breathing will be observed. During endemic disease the virus continually circulates through the herd infecting individual pigs within groups. Swine influenza viruses cause severe pneumonia on their own but when they are combined with other infections such as enzootic pneumonia caused by M. hyopneumoniae and PRRSV a chronic respiratory disease syndrome can develop.

**Enzootic pneumonia** is caused by **Mycoplasma hyopneumoniae.** It is widespread in pig populations and endemic in most herds throughout the world. It is transmitted either through the movement of the carrier pigs or by wind-borne infection for up to 2 - 3km (1 - 2 miles). The organism dies out quickly outside the pig, particularly when dried. It can however be maintained in

moist cool conditions for two to three days. It has a long incubation period of two to eight weeks before clinical symptoms are seen. As an uncomplicated infection in well-housed and managed pigs it is a relatively unimportant disease and has only mild effects on the pig. If EP is not present in the growing population then the effects of the other respiratory pathogens are very greatly reduced. It is therefore considered a prime organism that opens up the lung to other infections. **Acute disease** is normally only seen when EP is introduced into the herd for the first time. For a period of six to eight weeks after entry there may be severe acute pneumonia, coughing, respiratory distress, fever and high mortality across all ages of stock. This picture however is extremely variable and breakdowns are experienced when disease is mild or inapparent. **Chronic disease** is the normal picture where the organism has been present in the herd for some considerable time. Maternal antibody is passed via colostrum to the piglets and it disappears from seven to twelve weeks of age after which clinical signs start to appear.

# 3 Principle of the Test

The respiPig 1 real time RT-PCR contains specific primers and hydrolysis probes for the simultaneous detection of the RNA of the North American (NA) and European (EU) genotypes of PRRSV, the RNA of Flu A and the DNA of M. hyopneumoniae. Extracted RNA and DNA from swine blood, serum, tissue, bronchial swabs, saliva, bronchial lavage, semen, and cell culture supernatant samples can be used for analysis. The reverse transcription (RT) of viral RNA to cDNA and the subsequent amplification of virus specific fragments (PRRSV and Flu A) and the amplification of bacteria specific fragments (M. hyopneumoniae), respectively, are performed in a one-step RT-PCR. The amplification can be detected when specific probes are hydrolysed by the Polymerase. The emitted fluorescence signals are measured in the FAM (Flu A), Cy5 (PRRSV) and ROX (M. hyopneumoniae) channels.

Furthermore, the respiPig 1 real time RT-PCR contains a Control RNA, which is detected simultaneously. Added during the extraction, the Control RNA allows not only monitoring of RT-PCR inhibition but also detects possible mistakes during extraction. This greatly reduces the risk of false-negative results. The Control RNA can also be used solely as Internal Control by adding it directly to the Mastermix. The fluorescence signal of the Control RNA is measured in the VIC®/HEX/JOE™/TET channel.

# 4 Package Contents

The reagents supplied are sufficient for 32 or 96 reactions respectively.

Table 1: Components of the respiPig 1 real time RT-PCR Kit.

| Label | Lid Colour | Content 32 | Content 96 |
|---|---|---|---|
| Reaction Mix | yellow | 1 x 506 µl | 2 x 759 µl |
| Enzyme | blue | 1 x 6.4 µl | 1 x 19.2 µl |
| Positive Control (PRRSV/Flu A/ M. hyopneumoniae) | red | 1 x 50 µl | 1 x 100 µl |
| Negative Control | green | 1 x 50 µl | 1 x 100 µl |
| Control RNA | colourless | 1 x 160 µl | 2 x 240 µl |

# 5 Equipment and Reagents to be Supplied by User

- DNA/RNA isolation kit (e.g. NukEx Pure RNA/DNA, gerbion Cat. No. G05004 or NukEx Mag RNA/DNA, gerbion Cat. No. G05012)
- PCR grade Water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Table centrifuge
- Vortexer
- Real time PCR instrument
- Optical PCR reaction tubes with lid
- Optional: Liquid handling system for automation
- Optional: Colour Compensation Kit for use of respiPig 1 real time RT-PCR with LightCycler 480II, available from gerbion on request

# 6 Transport, Storage and Stability

The respiPig 1 real time RT-PCR Kit is shipped on dry ice or cool packs.
All components must be stored at maximum -18°C in the dark immediately after receipt. Do not use reagents after the date of expiry printed on the package. Up to 20 freeze and thaw cycles are possible.
For convenience, opened reagents can be stored at +2-8°C for up to 6 months.
Protect kit components from direct sunlight during the complete test run.

# 7 Important Notes

- The respiPig 1 real time RT-PCR must be performed by qualified personnel only.
- Good Laboratory Practice (GLP) has to be applied.
- Samples must always be regarded as potentially infectious material and all equipment used has to be treated as potentially contaminated.

# 8 General Precautions

- Stick to the protocol described in the Instruction for Use.
- Set up different laboratory areas for the preparation of samples and for the set up of the RT-PCR in order to avoid contaminations.
- Pipettes, tubes and other materials must not circulate between those different laboratory areas.
- Always use filter tips.
- Regularly decontaminate equipment and benches with ethanol-free decontaminant.
- Do not combine respiPig 1 real time RT-PCR Kit components of different lot numbers.

# 9 Sample Material

Starting material for the respiPig 1 real time RT-PCR is the nucleic acid isolated from clinical specimens (e.g. swine blood, serum, tissue, bronchial swabs, saliva, bronchial lavage, semen) and cell culture supernatant samples.

## 10 Sample Preparation

Commercial kits for RNA/DNA isolation such as the following are recommended:

- NukEx Pure RNA/DNA, gerbion Cat. No. G05004
- NukEx Mag RNA/DNA, gerbion Cat. No. G05012

**Important:** In addition to the samples always run a "water control" in your extraction. Treat this water control analogous to a sample.
Comparing the amplification of the Control RNA in the sample to the amplification of the Control RNA in the water control will give insights on possible inhibitions of the real time RT-PCR. Furthermore, possible contaminations during RNA extraction will be detectable.

Please note chapter ‚Control RNA'.

If the real time RT-PCR is not performed immediately, store extracted RNA/DNA according to the instructions given by the nucleic acid extraction kit's manufacturer.

## 11 Control RNA

A Control RNA is supplied and can be used as extraction control or only as inhibition control. This allows the user to control the nucleic acid isolation procedure and to check for possible real time RT-PCR inhibition.

a) Control RNA used as Extraction Control:
respiPig 1 real time RT-PCR Control RNA is added to the extraction.
Add 5 µl Control RNA per extraction (5 µl x (N+1)). Mix well. Perform the RNA isolation according to the manufacturer's instructions. Please follow protocol A.
The Control RNA must be added to the Lysis Buffer of the extraction kit.

b) Control RNA used as Internal Control of the real time PCR:
If only inhibitions of the real time RT-PCR will be checked please follow protocol B.

# 12 Real time RT-PCR

## 12.1 Important Points Before Starting:

- Please pay attention to chapter 7 ‚Important Notes'.
- Before setting up the real time RT-PCR familiarise yourself with the real time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the PCR set up.
- In every RT-PCR run one Positive Control and one Negative Control should be included.
- Before each use, all reagents - except the Enzyme - should be thawed completely at room temperature, thouroughly mixed, and centrifuged very briefly.

## 12.2 Procedure

If the Control RNA is used to control both, the real time RT-PCR and the RNA/DNA isolation procedure, please follow protocol A. If the Control RNA is solely used to detect possible inhibition of the real time RT-PCR, please follow protocol B.

Protocol A

**The Control RNA was added during RNA/DNA extraction (see chapter ‚Control RNA'). In this case, prepare the Master Mix according to Table 2**.

The Master Mix contains all of the components needed for RT-PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 2: Preparation of the Master Mix

| Volume per Reaction | Volume Master Mix |
|---|---|
| 15.8 µl Reaction Mix | 15.8 µl x (N+1) |
| 0.2 µl Enzyme | 0.2 µl x (N+1) |

Protocol B

**The Control RNA is used for the control of the real time RT-PCR only (see chapter ‚Control RNA'). In this case, prepare the Master Mix according to Table 3.**

The Master Mix contains all of the components needed for real RT-PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

**Important:** Dilute the Control RNA 1:10 in PCR-grade $dH_2O$ (e.g. 1 µl Control RNA + 9 µl PCR grade Water) before adding it to the Master Mix.

Table 3: Preparation of the Master Mix

| Volume per Reaction | Volume Master Mix |
|---|---|
| 15.8 µl Reaction Mix | 15.8 µl x (N+1) |
| 0.2 µl Enzyme | 0.2 µl x (N+1) |
| 0.2 µl Control RNA* **(diluted 1:10)** | 0.2 µl x (N+1)* |

*The increase in volume caused by adding the Control RNA is not taken into account when preparing the PCR assay. The sensitivity of the detection system is not impaired.

Protocol A and B: real time RT-PCR set up

- Place the number of optical PCR reaction tubes needed into the respective tray of the real time PCR instrument.
- Pipet **16 µl** of the Master Mix into optical PCR reaction tubes.
- Add **4 µl** of the eluates from the RNA/DNA isolation (including the eluate of the water control), the Positive Control, and the Negative Control to the corresponding optical PCR reaction tubes (Table 4).
- Close the optical PCR reaction tubes immediately after filling in order to reduce the risk of contamination.

Table 4: Preparation of the real time PCR

| Component | Volume |
|---|---|
| Master Mix | 16.0 µl |
| Sample | 4.0 µl |
| Total Volume | 20.0 µl |

## 12.3 Instrument Settings

For the real time RT-PCR use the thermal profile shown in Table 5.

Table 5: real time RT-PCR thermal profile

| Description | Time | Temperature | Number of Cycles |
|---|---|---|---|
| Reverse Transcription | 10 min | 50°C | 1 |
| Initial Denaturation | 1 min | 95°C | 1 |
| Amplification of cDNA | | | 45 |
| Denaturation | 10 sec | 95°C | |
| Annealing and Extension | 40 sec<br>Aquisition at the end of this step | 60°C | |

Dependent on the real time instrument used, further instrument settings have to be adjusted according to Table 6.

Table 6: Overview of the instrument settings required for the respiPig 1 real time RT-PCR Kit.

| Real time PCR Instrument | Parameter Reaction Mix | Detection Channel | Notes | | |
|---|---|---|---|---|---|
| | | | Colour Compensation required | | |
| | | | Melt Factor | Quant Factor | Max Integration Time (sec) |
| LightCycler 480II | Flu A | 465-510 | 1 | 10 | 1 |
| | M. hyopneumoniae | 533-610 | 1 | 10 | 2 |
| | Control RNA | 533-580 | 1 | 10 | 2 |
| | PRRSV EU/NA | 618-660 | 1 | 10 | 3 |
| Stratagene Mx3000P / Mx3005P | Flu A | FAM | Gain 8 | | |
| | M. hyopneumoniae | ROX | Gain 1 | | Reference Dye: None |
| | Control RNA | HEX | Gain 1 | | |
| | PRRSV EU/NA | Cy5 | Gain 4 | | |
| ABI 7500 | Flu A | FAM | | | |
| | M. hyopneumoniae | ROX | Option Reference Dye ROX: NO | | |
| | Control RNA | JOE | | | |
| | PRRSV EU/NA | Cy5 | | | |
| Rotor-Gene Q, Rotor-Gene 3000 Rotor-Gene 6000 | Flu A | Green | Gain 5 | | |
| | M. hyopneumoniae | Orange | Gain 5 | | |
| | Control RNA | Yellow | Gain 5 | | |
| | PRRSV EU/NA | Red | Gain 5 | | |
| mic qPCR Cycler | Flu A | Green | Gain 8 | | |
| | M. hyopneumoniae | Orange | Gain 10 | | |
| | Control RNA | Yellow | Gain 10 | | |
| | PRRSV EU/NA | Red | Gain 10 | | |

# 13 Data Analysis

The virus specific amplifications are measured in the FAM, ROX, and Cy5 channel. The amplification of the Control RNA is measured in the VIC®/HEX/JOE™/TET channel. The Positive Control contains in vitro transcripts of the respective nucleic acid sequences of PRRSV NA and PRRSV EU strains and Flu A and synthetic sequences of M. hyopneumoniae, respectively. For the Positive Control, signals in the FAM, ROX, and Cy5 channels must be detected. The interpretation of the test results is described in table 7.

Table 7: Interpretation of results

| Signal/Ct Values | | | | Interpretation |
|---|---|---|---|---|
| **FAM Channel** Flu A | **ROX Channel** M. hyopneumoniae | **Cy5 Channel** PRRSV EU/NA | **HEX Channel** Control RNA | |
| **positive** | negative | negative | positive or negative* | **Positive result, the sample contains Flu A-RNA.** |
| negative | **positive** | negative | positive or negative* | **Positive result, the sample contains M. hyopneumoniae-DNA.** |
| negative | negative | **positive** | positive or negative* | **Positive result, the sample contains PRRSV EU/NA-RNA.** |
| negative | negative | negative | ≤ 34** | **Negative result, the sample contains no Flu A-RNA, M. hyopneumoniae-DNA and PRRSV EU/NA-RNA.** |
| negative | negative | negative | negative or > 34** | **No diagnostic statement can be made.** The real time RT-PCR is either inhibited or errors occurred while RNA/DNA extraction. |

* A strong positive signal in the FAM, Cy5 and/or ROX can inhibit the IC. In such cases the result for the Control RNA can be neglegted.

** Depending on the PCR instrument and/or the chosen extraction method, the Ct values might be shifted. The water control can be used as reference. If the HEX Ct value of a sample differs a lot from the water control, partial inhibition has occurred, leading to false negative results in case of weak positive samples.

**Figure 1** and **Figure 2** show examples for positive and negative real time RT-PCR results.

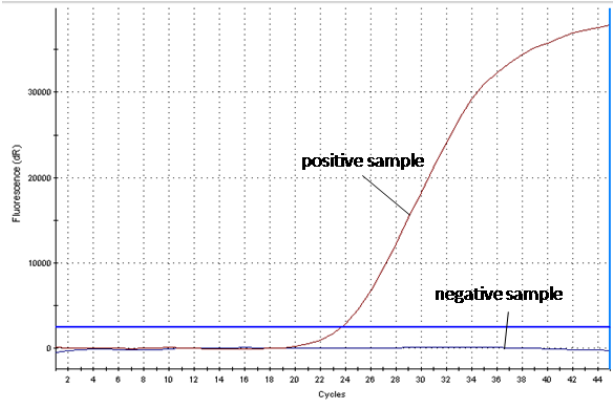


Figure 1: The positive sample shows specific amplification, whereas no fluorescence signal is detected in the negative sample.

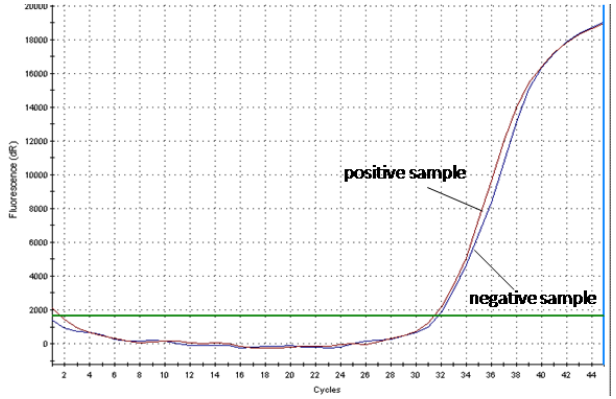


Figure 2: The positive sample as well as the negative sample show a signal in the Control RNA specific VIC®/HEX/JOE/TET channel. The amplification signal of the Control RNA in the negative sample shows, that the missing signal in the virus specific channels is not due to RT-PCR inhibition or failure of RNA/DNA isolation, but that the sample is a true negative.

# 14 Assay Validation

Set a threshold as follows:

### Negative Controls

All negative controls should be below the threshold. If there is a potential contamination (appearance of a curve in the negative control or a cluster of curves in specimens at high $C_T$ – for example above 36), results obtained are not interpretable and the whole run (including extraction) has to be repeated.

### Positive Controls

All the positive controls must show a positive (i.e. exponential) amplification curve. The positive controls must fall below a $C_T$ of 30.

### Internal Controls

All internal controls must show a positive (i.e. exponential) amplification curve. The internal control must fall below a $C_T$ of 34. If the internal control is above $C_T$ 34, this points to a purification problem or a strong positive sample that can inhibit the IC. In the latter case, the assay is valid. If a water control run is performed, the IC must fall below a $C_T$ of 34.

# 15 Troubleshooting

The following troubleshooting guide is included to help you with possible problems that may arise when performing a real time RT-PCR. If you have further questions, please do not hesitate to contact our scientists on info@gerbion.com.

| No fluorescence signal in the FAM, ROX, Cy 5 channel of the Positive Controls | |
|---|---|
| The selected channel for analysis does not comply with the protocol | Select the FAM channel for analysis of the Flu A specific amplification, the ROX channel for analysis of the M. hyopneumoniae specific amplification and the Cy5 channel for analysis of the PRRSV specific amplification. Select the VIC®/HEX/JOE™/TET channel for the amplification of the Control RNA. Due to amplification in the specific channels, amplification of the Internal Control can be inhibited in the Positive Control. |
| Incorrect configuration of the real time RT-PCR | Check your work steps and compare with “Procedure”. |
| The programming of the thermal profile is incorrect | Compare the thermal profile with the protocol in “Instrument Settings”. |

| | |
|---|---|
| Incorrect storage conditions for one or more kit components or kit expired | Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in "Transport, Storage and Stability". |
| **Weak or no signal of the Control RNA and simultaneous absence of a signal in the specific FAM channel, ROX channel or Cy5 channel.** | |
| real time RT-PCR conditions do not comply with the protocol | Check the real time RT-PCR conditions. |
| real time RT-PCR inhibited | Make sure that you use an appropriate isolation method (see "Sample Preparation") and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffer of the isolation kit has been completely removed. |
| RNA/DNA loss during isolation process | In case the Control RNA was added before extraction, the lack of an amplification signal can indicate that the RNA/DNA isolation was not successful. Make sure that you use an appropriate isolation method (commercial kits are recommended) and stick to the manufacturer's protocol. |
| Incorrect storage conditions for one or more components or kit expired | Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in "Transport, Storage and Stability". |
| **Detection of a fluorescence signal in the FAM channel, ROX channel or Cy5 channel of the Negative Control.** | |
| Contamination during preparation of the RT-PCR | Repeat the real time RT-PCR in replicates. If the result is negative in the repetition, the contamination occured when the samples were pipetted into the optical PCR reaction tubes. Make sure to pipet the Positive Control last and close the optical PCR reaction tube immediately after adding the sample. If the same result occurs, one or more of the kit components might be contaminated. Make sure that work space and instruments are decontaminated regularly. Use a new kit and repeat the real time RT-PCR. |

# 16 Kit Performance

## 16.1 Analytical Sensitivity

The limit of detection (LoD) of respiPig 1 real time RT-PCR was determined using serial dilutions of in vitro transcripts (PRRSV NA, PRRSV EU, Flu A) and a synthetic DNA-fragment of M. hyopneumoniae. A Stratagene Mx3005 real time PCR instrument was used for amplification and detection.

Table 8: Samples tested for the validation of the sensitivity of respiPig 1 real time RT-PCR.

| PRRSV NA | Copies per Reaction | Expected Result | respiPig 1 |
|---|---|---|---|
| | 1.000.000 | positive | positive |
| | 100.000 | positive | positive |
| | 10.000 | positive | positive |
| | 1.000 | positive | positive |
| | 100 | positive | positive |
| | 10 | positive | positive |

| PRRSV EU | Copies per Reaction | Expected Result | respiPig 1 |
|---|---|---|---|
| | 1.000.000 | positive | positive |
| | 100.000 | positive | positive |
| | 10.000 | positive | positive |
| | 1.000 | positive | positive |
| | 100 | positive | positive |
| | 10 | positive | positive |

| Flu A | Copies per Reaction | Expected Result | respiPig 1 |
|---|---|---|---|
| | 100.000 | positive | positive |
| | 10.000 | positive | positive |
| | 1.000 | positive | positive |
| | 100 | positive | positive |
| | 10 | positive | positive |

| M. hyopneumoniae | Copies per Reaction | Expected Result | respiPig 1 |
|---|---|---|---|
| | 1.000.000 | positive | positive |
| | 100.000 | positive | positive |
| | 10.000 | positive | positive |
| | 1.000 | positive | positive |
| | 100 | positive | positive |
| | 10 | positive | positive |

## 16.2 Analytical Specificity

The specificity of the respiPig 1 real time RT-PCR was evaluated by in silico analysis and by amplification of RNA and DNA of other relevant viruses and bacteria found in clinical samples.

The respiPig 1 real time RT-PCR showed positive results for the samples containing RNA from PRRSV NA, PRRSV EU, Flu A and DNA from M. hyopneumoniae, whereas samples containing other pathogens were reliably tested negative. The results are shown in table 9.

Table 9: Bacterial and viral pathogens tested for the determination of the analytical specificity of respiPig 1 real time RT-PCR.

| Sample | Status | respiPig 1 real time Rt-PCR | | |
|---|---|---|---|---|
| | | FAM | ROX | Cy5 |
| 11/ ASFV Niederlande DNA | ASFV pos. | negative | negative | negative |
| 12/ ASFV"Estland" DNA | ASFV pos. | negative | negative | negative |
| 13/ ASFV"Kenia" DNA | ASFV pos. | negative | negative | negative |
| 14/ ASFV"Sardinien" DNA | ASFV pos. | negative | negative | negative |
| 20/ CSFV"Litauen", RNA | CSFV pos. | negative | negative | negative |
| CSF0751 (RV213/18) | CSFV pos. | negative | negative | negative |
| CSF0679 (RV213/18) | CSFV pos. | negative | negative | negative |
| CSF0383 (RV213/18) | CSFV pos. | negative | negative | negative |
| CSF0932 (RV213/18) | CSFV pos. | negative | negative | negative |
| CSF0306 (RV213/18) | CSFV pos. | negative | negative | negative |
| CSF0653 (RV213/18) | CSFV pos. | negative | negative | negative |
| QCMD ADVDNA14-06 (RV134/14) | *Adenovirus* | negative | negative | negative |
| QCMD BPDNA 14-08 (RV127/14) | *Bordetella pertussis* | negative | negative | negative |
| Instand RV 370_15 Intern 141/15 Probe 370055 | *Influenza B* | negative | negative | negative |
| QCMD MTBDNA14-01 (RV 124/14) | *Mycobacterium tuberculosis* | negative | negative | negative |
| Instand RV 541/2015 (RV 151/15) Probe 1515413 | *Mycoplasma pneumoniae* | negative | negative | negative |

| | | | | |
|---|---|---|---|---|
| ATCC-VR-1360 | *Chlamydia pneumoniae* | negative | negative | negative |
| QCMD CVRNA 14-02 | *Coronavirus* | negative | negative | negative |
| ATCC 15597 | *Escherichia coli* | negative | negative | negative |
| PRRSV (EU) Lelystad Virus | *PRRSV EU* | negative | negative | **positive** |
| PRRSV (NA) ATCC VR 2332 | *PRRSV NA* | negative | negative | **positive** |
| Instand RV 370_15 Intern 141/15 Probe 370056 | *Influenza A* | **positive** | negative | negative |
| M. hyopneumoniae (CVUA, Stuttgart) | *M. hyopneumoniae* | negative | **positive** | negative |

## 16.3 Diagnostic Sensitivity and Specificity

During the validation study of the respiPig 1 real time RT PCR positive and negative clinical samples, previously characterized using other real time RT-PCR, were tested. The diagnostic sensitivity was found to be 100% and the diagnostic specificity 100%.

Table 10: Overview of the amount of samples tested and the diagnostic sensitivity and specificity

| | **positive samples** | **negative samples** |
|---|---|---|
| respiPig 1 positive | | |
| pan PRRSV | 17 | 0 |
| M. hyopneumoniae | 12 | 0 |
| Influenzavirus A | 4 | 0 |
| respiPig 1 negative | | |
| pan PRRSV | 0 | 18 |
| M. hyopneumoniae | 0 | 23 |
| Influenzavirus A | 0 | 31 |
| **Sensitivity** | 100% | |
| **Specificity** | 100% | |

## 17 Abbreviations and Symbols

| | |
|---|---|
| cDNA | complementary Deoxyribonucleid Acid |
| RNA | Ribonucleid Acid |
| PCR | Polymerase Chain Reaction |
| RT | Reverse Transcription |
| ASFV | African Swine Fever Virus |
| CSFV | Classical Swine Fever Virus |
| Flu A | Influenza Viruses Type A |
| REACTION MIX | Reaction Mix |
| ENZYME | Enzyme |
| CONTROL + | Positive Control |
| CONTROL − | Negative Control |
| CONTROL RNA IC | Control RNA |

| | |
|---|---|
| REF | Catalog number |
| Σ | Contains sufficient for <n> test |
| -18°C | Upper limit of temperature |
| | Manufacturer |
| | Use by YYYY-MM-DD |
| LOT | Batch code |
| CONT | Content |
| | Consult instructions for use |

## 18 Literature

[1] Moh A. Alkhamis, Andreia G. Arruda, Robert B. Morrison, Andres M. Perez. Novel approaches for Spatial and Molecular Surveillance of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in the United States, Nature.com., Scientific Reports volume 7, Article number: 4343 (2017)

[2] Pileri, E; Mateu, E (28 October 2016). "Review on the transmission porcine reproductive and respiratory syndrome virus between pigs and farms and impact on vaccination". Veterinary research. 47 (1): 108

[3] Information on Swine Influenza/Variant Influenza Virus. www.cdc.gov/flu/swineflu/index.htm. Page last updated: August 6, 2018

[4] Maes D., Segales J., Meyns T., Sibila M., Pieters M., Haesebrouck F. Control of *Mycoplasma hyopneumoniae* infections in pigs (2008) Veterinary Microbiology, 126 (4), pp. 297-309.