BIOVET
A DIVISION OF ANTECH®

# Porcine Epidemic Diarrhea Virus Antibody Test Kit, ELISA

## Swinecheck® PEDV

Product code: TRM-556 (2 plates)
TRM-558 (5 plates)

- ✓ **A sensitive and specific ELISA kit**
- ✓ **Approved by the Canadian Food Inspection Agency**
- ✓ **Available in two different formats**

### INTRODUCTION

Porcine epidemic diarrhea (PED) is a swine disease characterized by acute diarrhea and vomiting. The disease is very contagious and affects pigs of all ages. The mortality may reach 100% in young piglets but is negligible in mature pigs.

PED is caused by a coronavirus distinct from the other porcine coronaviruses more especially the transmissible gastroenteritis virus (TGEV). PED was first reported in Europe in the late ’60 and is now prevalent in China, the Asian South East and more recently in the USA.

PED diagnosis relies on the demonstration of PEDV nucleic acids or antigens in various specimens (e.g. intestines, feces or oral fluids) or of PEDV antibodies in serum. Several serological tests have been developed to detect PEDV antibodies (e.g. IFA, ELISA). Enzyme-Linked Immuno Assays (ELISA) are easy to perform and to be automatized.

At Biovet we have developed an indirect ELISA using a recombinant nucleoprotein (NP) protein as antigen. NP provides an attractive target for the development of serological assays. Indeed, the NP gene is highly conserved (94.7−97.7 %). In addition, the NP is the most abundant viral protein expressed in infected cells. In contrast, the spike (S) protein demonstrates a greater range of genetic heterogeneity including insertions and deletions.

Our PEDV-NP antigen is a recombinantly expressed full length North American PEDV-NP. The NP open reading frame (ORF) of a PEDV US original strain was cloned into *E. coli*. The recombinant protein was expressed and purified. Its identity was confirmed via Western Blotting using convalescent porcine sera, a 6X histidine-specific mAb (Novagen, Madison, WI) and a PEDV-NP specific mAb.

### INTENDED USE

Swinecheck® PED indirect is an immunoenzymatic assay for the detection of antibodies to porcine epidemic diarrhea virus (PEDV) in porcine serum sample. It has been approved by the Canadian Food Inspection Agency (CCVB file number 780DR/P1.0/D10) in 2017.



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## PRINCIPLE OF THE TEST

### a. Assay description

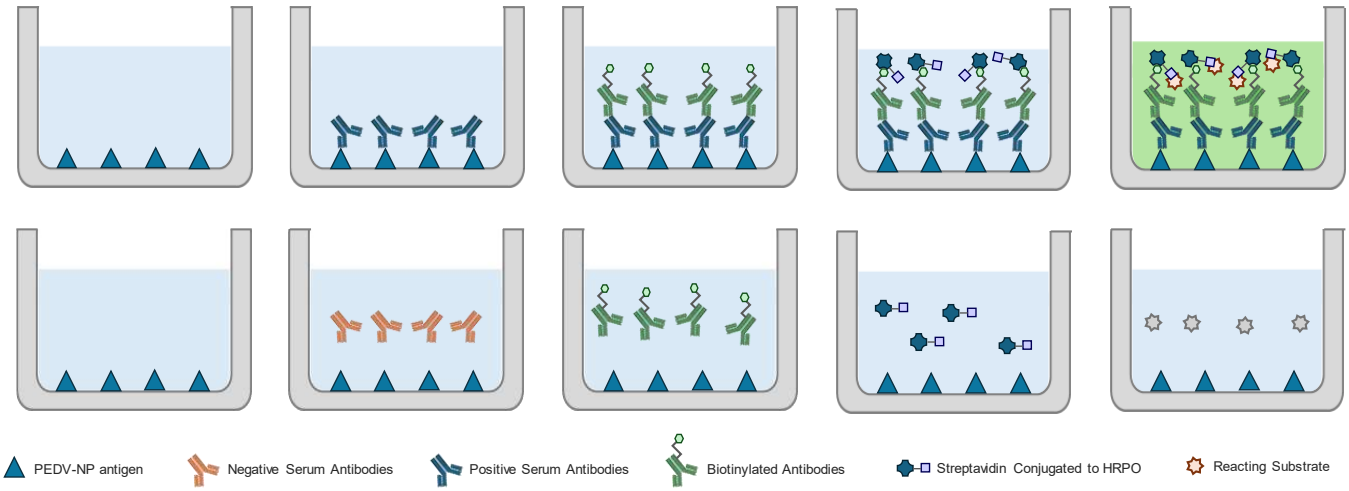
1. The wells are coated with a recombinant nucleoprotein (NP) of PEDV.

2. Samples are added and incubated for 1 hour at 23 ± 2°C. Each well is then washed carefully to remove unbound material.

3. Biotinylated polyclonal antibodies targeting porcine IgG (Conjugate A) are added and incubated for 1 hour at 23 ± 2°C. Washing steps follow.

4. Streptavidin conjugated to horseradish peroxidase (HRPO) (Conjugate B) is added and incubated for 1 hour at 23 ± 2°C. Washing steps follow.

5. The substrate is added and incubated for 10 min at 23 ± 2°C. It will react with HRPO if present. The reaction is stopped, and OD values are measured.



**High OD values resulting in a high S/P ratio.**

**Low OD values resulting in a low S/P ratio.**




### b. Results interpretation

OD values are used to calculate the S/P ratio as follow:

$$\text{S/P RATIO} = \frac{OD_{sample} - mOD_{neg}}{mOD_{pos} - mOD_{neg}}$$

The status of a test sample is determined with the S/P ratio

| Result | S/P ratio |
|---|---|
| Negative | < 0.40 |
| Positive | ≥ 0.40 |

The following criteria must be met in order to validate the test:

- Mean OD of the Negative Control must be lower than 0.400.
- Corrected OD of the Positive Control must be greater than 0.700.



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## TECHNICAL DATA

### a. Sensitivity & Specificity

625 serum samples were used for evaluating the performance of the test under evaluation:

- 125 samples originating from several Canadian and US herds infected with PEDV
- 500 samples originating from numerous Canadian herds free from PEDV

The PEDV status of the herds was established based on their clinical history and the detection (or absence of detection) of PEDV using the EZ-PEDV/TGEV/PDCoV qPCR kit (Tetracore, Rockville, USA).

Samples were also tested for PEDV antibodies using an immunofluorescence assay (IFA) and/or a virus neutralization test (VNT) at the Department of Veterinary and Biomedical Sciences of the South Dakota State University (SDSU) (Dr Eric Nelson).

| A | IFA + | IFA - | Total |
|---|---|---|---|
| **Biovet +** | 110 | 22 | **132** |
| **Biovet -** | 0 | 493 | **493** |
| **Total** | **110** | **515** | **625** |

| Statistic | Value | 95% CI |
|---|---|---|
| **Relative sensitivity** | 100.0% | 96.70% to 100.0% |
| **Relative specificity** | 95.73% | 93.60% to 97.30% |
| **Kappa value** | 0,874 | |

**The Swinecheck PEDV kit showed excellent sensitivity, specificity, and a strong agreement with the reference test.**

### b. Repeatability

The intra-lot (A) and inter-lot (B) repeatability was evaluated using a panel of 6 samples. Each sample was tested 3 times in one serial, 3 times in 3 distinct serials. Mean and standard deviation of OD values were used to calculate the Coefficient of Variation (CV).

**A**

| Sample | Plate 1 | Plate 2 | Plate 3 | Mean ± SD | CV (%) |
|---|---|---|---|---|---|
| CQ 606 | 0,224 | 0,238 | 0,213 | 0,225 ± 0,013 | **5,6** |
| CQ 608 | 0,129 | 0,133 | 0,116 | 0,126 ± 0,009 | **7,1** |
| CQ 618 | 2,882 | 3,175 | 2,975 | 3,011 ± 0,150 | **5,0** |
| CQ 619 | 1,665 | 1,714 | 1,595 | 1,658 ± 0,060 | **3,6** |
| CQ 621 | 1,985 | 2,025 | 1,969 | 1,993 ± 0,029 | **1,4** |
| CQ 622 | 3,388 | 3,656 | 3,589 | 3,544 ± 0,139 | **3,9** |

**B**

| Sample | Serial A | Serial B | Serial C | Mean ± SD | CV (%) |
|---|---|---|---|---|---|
| CQ 606 | 0,179 | 0,224 | 0,180 | 0,194 ± 0,026 | **13,2** |
| CQ 608 | 0,109 | 0,129 | 0,109 | 0,116 ± 0,012 | **10,0** |
| CQ 618 | 2,554 | 2,882 | 2,550 | 2,662 ± 0,191 | **7,2** |
| CQ 619 | 1,257 | 1,665 | 1,442 | 1,455 ± 0,204 | **14,0** |
| CQ 621 | 1,515 | 1,985 | 1,634 | 1,711 0,244 | **14,3** |
| CQ 622 | 3,329 | 3,388 | 2,906 | 3,208 ± 0,263 | **8,2** |

**In both intra- and inter-lot studies, the OD values of all tested samples had a CV lower than 15%.**



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### c. Stability

The stability of the kit was evaluated using 3 kits from different serials that have been stored at 4 - 8°C for 13 to 17 months.

| Samples | Reactivity | Serial A | | Serial B | | Serial C | |
|---|---|---|---|---|---|---|---|
| | | 0 mo. | 13 mo. | 0 mo. | 15 mo. | 0 mo. | 17 mo. |
| Neg. Ctrl. | Negative | 0,05 | 0,06 | 0,06 | 0,07 | 0,08 | 0,07 |
| Pos. Ctrl. | Positive | 1,00 | 1,00 | 1,00 | 1,00 | 1,00 | 1,00 |
| CQ 606 | Negative | 0,11 | 0,12 | 0,14 | 0,15 | 0,14 | 0,17 |
| CQ 607 | Negative | 0,07 | 0,09 | 0,08 | 0,11 | 0,08 | 0,09 |
| CQ 608 | Negative | 0,06 | 0,09 | 0,08 | 0,11 | 0,09 | 0,11 |
| CQ 609 | Negative | 0,09 | 0,14 | 0,14 | 0,14 | 0,12 | 0,12 |
| CQ 613 | Weak Positive | 0,58 | 0,62 | 0,93 | 0,76 | 0,71 | 0,70 |
| CQ 619 | Positive | 0,88 | 0,82 | 1,05 | 0,94 | 0,93 | 1,07 |
| CQ 621 | Positive | 1,03 | 0,92 | 1,26 | 1,12 | 1,06 | 1,10 |
| CQ 622 | Positive | 1,91 | 1,90 | 2,23 | 2,42 | 2,49 | 2,07 |

**The S/P ratios for each sample remained stable throughout the study, and no changes in result outcomes were observed showing the kit's great stability.**

## CONCLUSION

The Swinecheck® PEDV is suitable to detect the antibodies from the Porcine Epidemic Diarrhea Virus. It is stable, reproducible, sensitive and specific.

## KIT COMPOSITION

| Components | Quantity TRM-556 | Quantity TRM-558 |
|---|---|---|
| 12 strips of 8 wells coated with PEDV Ag | 2 | 5 |
| Ready-to-use Positive Control | 2.5 mL | 6 mL |
| Ready-to-use Negative Control | 2.5 mL | 6 mL |
| Concentrated Conjugate A | Variable | Variable |
| Concentrated Conjugate B | Variable | Variable |
| Ready-to-use Dilution Buffer | 3 x 100 mL | 5 x 120 mL |
| Concentrated Wash Solution (10X) | 2 x 100 mL | 4 x 100 mL |
| Ready-to-use Substrate | 25 mL | 60 mL |
| Ready-to-use Stop Solution | 25 mL | 60 mL |


**CFIA-licensed FEO; 780DR/P1.0/D10**

**For more info, contact us at: 1-888-824-6838, option 3**
**order@biovet-inc.com**.

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4375, av. Beaudry, Saint-Hyacinthe QC J2S 8W2, Canada