



TGEV-PRCV Antibody Differentiating Test Kit, ELISA

Swinecheck® TGEV-PRCV Recombinant

Product code: TRM-535 (2 plates)
TRM-552 (5 plates)

- ✓ **Distinguishes TGEV from other PRCVs**
- ✓ **A shelf life of 24 months**
- ✓ **Approved by the Canadian Food Inspection Agency**
- ✓ **Available in two different formats**

INTRODUCTION

Transmissible gastroenteritis (TGE) is a highly contagious enteric disease of swine caused by TGEV. TGEV affects swine of all ages. It multiplies in the digestive and respiratory tracts and causes vomiting and diarrhea. The mortality rate in piglets under 2 weeks of age is near 100%. In older pigs, the disease is milder and can even occasionally go unnoticed.

PRCV is a respiratory variant of TGEV which has lost the ability to colonize the digestive tract. PRCV infects swine of all ages and can cause benign respiratory infections. Its close relationship with TGEV complicates the diagnosis of TGE.



A fast and accurate diagnosis of TGE is essential to prevent dissemination of the disease. Diagnosis is mostly based on viral identification. However detection of antibodies is very useful in older pigs that do not always display typical signs of disease. Serological tests are also regularly required for commercial exchanges.

As antibodies against TGEV and PRCV show complete cross-reaction by neutralization, the differentiation of antibodies aimed against these two viruses is impossible by seroneutralization testing. However, the use of monoclonal antibodies in a competitive ELISA assay allows the detection and differentiation of infections caused by these two viruses. The specificity of the assay is improved by using a recombinant protein as antigen.

INTENDED USE

TGEV-PRCV Antibody Differentiating Test Kit developed by Biovet is an ELISA which allows the detection and differentiation of antibodies against transmissible gastroenteritis virus (TGEV) and other porcine respiratory coronavirus (PRCV) in swine serum. It has been approved by the Canadian Food Inspection Agency (CCVB file number 880DR/T10.1/D10) in 2010.

PRINCIPLE OF THE TEST

a. Assay description

Porcine serum samples and the controls are incubated in two wells coated with recombinant protein S of TGEV. The antibodies (Ab) specific to TGEV and PRCV possibly present in positive serum samples will bind to the protein in the wells.

After several washes to eliminate unbound substances, a monoclonal antibody (mAb) coupled to an enzyme (conjugate) and specific to TGEV is added into the first well (odd column), and a conjugate specific to both TGEV and PRCV is added into the second well (even column). The mAbs bind to the protein sites that have not been bound by the serum Ab present in positive samples.

After incubation, the excess of mAbs is eliminated by a second wash and their attachment is revealed with a chromogenous substrate. Following this incubation, the enzymatic portion of the conjugate, if present, reacts with the substrate and a blue color develops.

The reaction is then stopped (the color changes from blue to yellow) and the optical densities (OD) are read at 450nm. The intensity of the color allows the determination of the type of sample tested.

The Percent Inhibition (PI) is calculated as follow, using the OD measured for each sample.

$$\text{PI TGEV (\%)} = \left[1 - \left(\frac{\text{OD}_{\text{sample}}}{\text{mOD}_{\text{negTGEV}}} \right) \right] \times 100 \quad \text{PI PRCV (\%)} = \left[1 - \left(\frac{\text{OD}_{\text{sample}}}{\text{mOD}_{\text{negPRCV}}} \right) \right] \times 100$$

b. Results interpretation

The status of a test sample is determined with the PI.

TGEV	PI (%)	Interpretation
	TGEV / PRCV	
0.00 - 44.99 %	0.00 - 39.99 %	Negative for TGEV and PRCV
45.00 - 54.99 %	40.00 - 100.00 %	Suspect* for TGEV
55.00 - 100.00 %	40.00 - 100.00 %	Positive for TGEV
0.00 - 44.99 %	40.00 - 100.00 %	Negative for TGEV; Positive for PRCV
45.00 - 100.00 %	0.00 - 39.99 %	Invalid**

* A second serum sample should be collected 2 weeks later and retested

** The sample must be retested.

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

	OD values	
	with TGEV-Ab Conjugate	with TGEV/PRCV-Ab Conjugate
TGEV-Ab Positive Control	< 0.300	< 0.300
PRCV-Ab Positive Control	> 0.700	< 0.300
Negative Control (mOD)	> 0.900	> 0.900

TECHNICAL DATA

a. Sensitivity & Specificity

Two hundred and ninety-two (292) serum samples were used for evaluating the relative sensitivity & specificity of the test. Samples were characterized using virus neutralization (A). They were also tested using a commercial ELISA (B) (SVANOVIR® TGEV/PRCV-Ab). Samples are described below.

- 106 samples originated from herds free from both viruses (15 herds).
- 87 samples originated from herds infected with PRCV but free from TGEV (16 herds).
- 90 samples originated from herds that had been infected with the TGEV (8 herds).
- 9 samples originated from pigs that had been vaccinated with a modified live virus vaccine (ProSystem TGE/Rota®, Intervet) (1 case). These pigs were free from PRCV before vaccination.

A	VN + (TGEV-PRCV)	VN –	Total
Biovet + (TGEV)	82	0	82
Biovet + (PRCV)	104	0	104
Biovet –	0	106	106
Total	186	106	292

B	TGEV positive herds		PRCV positive herds	
	Biovet	Commercial kit	Biovet kit	Commercial kit
TGEV +	76	50	0	2
PRCV +	14	36	87	82
Negative	0	1	0	3
Invalid	0	3	0	0
Total	90	90	87	87

Suspect samples for TGEV were considered positive. Virus neutralization did not differentiate TGEV from other PRCVs. Biovet's Swinecheck TGEV-PRCV kit detected all positive samples by virus neutralization. Biovet kit showed an excellent agreement with the TGEV-positive herd (76/90) compared to the other commercial kit (50/90). Moreover, no false positive for TGEV were observed in the PRCV-positive herd, demonstrating the kit's high efficiency in differentiating antibodies against the virus from antibodies directed against other PRCVs.

b. Repeatability

The intra-plate (A), inter-plate (B) and inter-lot (C) repeatability was evaluated using 8 samples. Each sample was tested 3 times in one plate of a serial, 3 times in 3 plates of a serial and 3 times in 3 distinct serials. Mean and standard deviation of OD values were used to calculate the Coefficient of Variation (CV).

A – Intra-plate study (3n)

Sample	TGEV-Ab Conjugate		PRCV-Ab Conjugate	
	Mean ± SD	CV (%)	Mean ± SD	CV (%)
Negative	1,374 ± 0,105	7,6	1,672 ± 0,094	5,6
Negative	1,350 ± 0,040	3,0	1,741 ± 0,056	3,2
PRCV Weak Positive	0,996 ± 0,042	4,2	0,599 ± 0,005	0,8
PRCV Positive	1,065 ± 0,051	4,8	0,105 ± 0,006	5,4
PRCV Positive	1,018 ± 0,046	4,5	0,190 ± 0,005	2,7
TGEV Weak Positive	0,847 ± 0,063	7,5	0,288 ± 0,002	0,5
TGEV Weak Positive	0,681 ± 0,021	3,1	0,187 ± 0,003	1,6
TGEV Positive	0,212 ± 0,025	12,0	0,172 ± 0,025	14,3

B – Inter-plate study (3n)

Sample	TGEV-Ab Conjugate		PRCV-Ab Conjugate	
	Mean ± SD	CV (%)	Mean ± SD	CV (%)
Negative	1,457 ± 0,034	2,3	1,710 ± 0,053	3,1
Negative	1,393 ± 0,080	5,8	1,741 ± 0,025	1,4
PRCV Weak Positive	0,950 ± 0,031	3,3	0,623 ± 0,022	3,6
PRCV Positive	1,030 ± 0,041	3,9	0,095 ± 0,006	6,5
PRCV Positive	0,977 ± 0,058	5,9	0,221 ± 0,029	13,1
TGEV Weak Positive	0,835 ± 0,013	1,5	0,305 ± 0,023	7,7
TGEV Weak Positive	0,646 ± 0,012	1,8	0,189 ± 0,010	5,1
TGEV Positive	0,201 ± 0,018	8,9	0,162 ± 0,009	5,4

C – Inter-lot study (3n)

Sample	TGEV-Ab Conjugate		PRCV-Ab Conjugate	
	Mean ± SD	CV (%)	Mean ± SD	CV (%)
Negative	1,393 ± 0,112	8,0	1,514 ± 0,141	9,3
Negative	1,377 ± 0,078	5,6	1,553 ± 0,162	10,5
PRCV Weak Positive	0,970 ± 0,055	5,7	0,610 ± 0,086	14,1
PRCV Positive	1,044 ± 0,059	5,6	0,100 ± 0,005	4,5
PRCV Positive	1,069 ± 0,067	6,3	0,191 ± 0,011	5,6
TGEV Weak Positive	0,820 ± 0,009	1,0	0,269 ± 0,019	7,1
TGEV Weak Positive	0,696 ± 0,035	5,1	0,180 ± 0,005	2,9
TGEV Positive	0,198 ± 0,031	15,9	0,156 ± 0,003	1,6

The optical density of all samples tested showed a coefficient of variation of less than 15% for both intra- and inter-plate analyses. The inter-lot comparison also showed high reproducibility, with only one sample having a CV greater than 15% (15.9) and this sample had a allow SD. These data clearly demonstrate the high reproducibility of the Swinecheck TGEV-PRCV kit.

c. Stability

Nine samples, with different reactivity were selected. Each sample was tested with 3 distinct lots of the kit at different time points (0, 12, 18, 24 months) postproduction. The Percent Inhibition (PI) was calculated as described in the introduction. The kits used for the stability tests were stored at 2-8°C until final testing.

Serial A

Samples	Reactivity	TGEV Plates				PRCV Plates			
		0 mo.	12 mo.	18 mo.	24 mo.	0 mo.	12 mo.	18 mo.	24 mo.
Sample 132	Negative	1,6	2,3	12,9	14,7	15,1	6,8	12,9	8,9
Sample 133	Negative	0,4	1,5	12,1	1,9	0,0	3,4	0,0	0,0
Sample 135	Negative	4,8	0,0	0,0	0,0	0,0	0,0	1,2	0,0
Sample 145	PRCV Positive	18,9	21,7	33,1	30,3	92,2	92,3	88,5	88,2
Sample 150	PRCV Positive	31,6	33,9	41,0	23,3	94,3	93,7	92,6	88,9
Sample 233	PRCV Positive	34,1	32,3	24,8	5,6	81,9	82,6	81,2	74,1
Sample 136	TGEV Positive	91,4	88,3	89,5	84,7	95,9	94,4	93,8	91,3
Sample 138	TGEV Positive	81,0	74,1	77,0	74,0	96,0	94,8	93,9	91,4
Sample 228	TGEV Positive	86,5	83,4	83,3	84,5	89,7	90,9	87,9	84,5

Serial B

Samples	Reactivity	TGEV Plates				PRCV Plates			
		0 mo.	12 mo.	18 mo.	24 mo.	0 mo.	12 mo.	18 mo.	24 mo.
Sample 132	Negative	15,1	6,8	12,9	8,9	0,0	16,6	5,5	22,3
Sample 133	Negative	5,6	0,0	10,4	13,2	0,0	8,2	0,0	12,2
Sample 135	Negative	11,7	4,0	9,1	0,3	0,0	0,0	0,4	0,0
Sample 145	PRCV Positive	23,9	33,2	25,1	25,2	92,4	92,4	91,5	88,1
Sample 150	PRCV Positive	30,8	35,8	37,3	37,7	94,3	93,1	93,7	91,3
Sample 233	PRCV Positive	38,5	22,7	27,7	38,2	79,5	81,8	83,4	83,0
Sample 136	TGEV Positive	92,5	89,9	87,7	89,3	96,2	94,4	94,5	92,8
Sample 138	TGEV Positive	83,3	80,3	76,1	79,5	96,2	94,8	94,7	92,8
Sample 228	TGEV Positive	87,4	86,0	84,2	84,6	90,7	89,4	90,0	89,0

Serial C

Samples	Reactivity	TGEV Plates				PRCV Plates			
		0 mo.	12 mo.	18 mo.	24 mo.	0 mo.	12 mo.	18 mo.	24 mo.
Sample 132	Negative	13,9	10,0	12,8	15,7	11,5	11,6	14,1	11,1
Sample 133	Negative	4,4	2,2	1,8	9,4	2,9	14,7	12,5	11,1
Sample 135	Negative	0,0	11,0	0,0	10,1	5,6	8,1	0,3	6,5
Sample 145	PRCV Positive	32,8	29,9	28,9	36,8	87,3	87,2	87,4	88,7
Sample 150	PRCV Positive	32,6	36,4	32,9	40,1	92,9	93,1	92,9	93,7
Sample 233	PRCV Positive	32,2	0,0	18,3	27,1	75,7	75,6	72,4	75,5
Sample 136	TGEV Positive	91,9	92,4	91,2	91,1	95,4	95,8	95,2	95,0
Sample 138	TGEV Positive	80,8	80,0	78,1	79,1	95,7	95,4	95,4	95,7
Sample 228	TGEV Positive	90,3	88,6	88,4	89,7	84,1	85,3	85,0	86,4

The PI of each sample remained stable over time for all three series. TGEV and PRCV positive samples remained positive throughout the study, and no false positives occurred among negative samples including PRCV positives tested in TGEV plates.

CONCLUSION

The Swinecheck® TGEV-PRCV kit has demonstrated excellent performances regarding repeatability and reproducibility. The relative sensitivity, relative specificity and agreement with the “gold standard” were almost perfect and the stability study showed that the kit is stable for 24 months when stored at 2-8°C.

KIT COMPOSITION

Components	Quantity TRM-535	Quantity TRM-552
12 strips of 8 wells coated with recombinant protein S of TGEV	2	5
Ready-to-use TGEV-Ab Positive Control	2 mL	6 mL
Ready-to-use PRCV-Ab Positive Control	2 mL	6 mL
Ready-to-use Negative Control	2.5 mL	6 mL
Ready-to-use Sample Dilution Buffer (green)	30 mL	75 mL
Ready-to-use TGEV-Ab Conjugate (blue)	11 mL	30 mL
Ready-to-use TGEV/PRCV-Ab Conjugate (red)	11 mL	30 mL
Concentrated Wash Solution (10X)	125 mL	2 x 125 mL
Ready-to-use Substrate	25 mL	60 mL
Ready-to-use Stop Solution	25 mL	60 mL



CFIA-licensed; 880DR/T10.1/D10

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