Transmissible Gastroenteritis Virus Antibody Test Kit, ELISA

Swinecheck® TGEV Recombinant

Product code: TRM-534 (2 plates)
TRM-539 (5 plates)

- ✓ **A sensitive and specific ELISA kit**
- ✓ **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.

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.

Antibodies against TGEV can be detected using the virus neutralization assay (VN). However, the VN test requires cell culture facilities and the use of infectious TGEV which limits its use to specialized laboratories.

Moreover, it is very time consuming and results are available after several days only. By contrast, enzyme-linked immunosorbent assays (ELISA) can be run in most laboratories and results are available within a few hours.

Use of monoclonal antibodies specific to TGEV in a blocking ELISA assay allows the detection of TGEV antibodies whereas antibodies directed against porcine respiratory coronavirus (PRCV), a closely related swine coronavirus, are not detected. The specificity of the assay is improved by using a recombinant protein as antigen.

INTENDED USE

Transmissible Gastroenteritis Virus Antibody Test Kit developed by Biovet is an ELISA which allows the detection of antibodies against TGEV in swine serum. It has been approved by the Canadian Food Inspection Agency (CCVB file number 880DR/T5.0/D10) in 2013.

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PRINCIPLE OF THE TEST

a. Assay description

Porcine serum samples as well as the controls are incubated in wells coated with recombinant protein S of TGEV. The antibodies (Ab) specific to TGEV 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. 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.

$$PI\ (\%) = 100 - \left(\frac{OD_{sample}}{\text{mean } OD_{neg}} \right) \times 100$$

b. Results interpretation

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

Result	PI (%)
Negative	0.00 - 44.99 %
Suspect*	45.00 - 54.99%
Positive	55.00 - 100.00%

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

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

- Mean OD of the Negative Control must be higher than 0.900.
- Mean OD of the Positive Control must be lower than 0.300.

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TECHNICAL DATA

a. Sensitivity & Specificity

One hundred and ninety-six (196) serum samples were used for evaluating the relative sensitivity & specificity of the test. Samples were characterized using virus neutralization (A) against porcine respiratory coronavirus (including, but not exclusive to TGEV). They were also tested using a commercial ELISA (SVANOVIR® TGEV/PRCV-Ab) (B). Samples are described below.

- 106 samples originated from herds free from coronavirus (15 herds).
- 90 samples originated from herds that had been infected with the TGEV (8 herds).

A	VN + (PRCV)	VN – (PRCV)	Total
Biovet + (TGEV)	76	0	**76**
Biovet – (TGEV)	14	106	**120**
Total	**90**	**106**	**196**

B	Biovet kit	Commercial kit
TGEV +	76	50
PRCV +	14	36
Negative	0	1
Unconclusive	0	3
Total	**90**	**90**

Suspect samples for TGEV were considered positive. Virus neutralization did not differentiate TGEV from other PRCVs. The 14 samples negative for TGEV but positive by serum neutralization tested positive for PRCV using Biovet's Swinecheck TGEV-PRCV kit. The Swinecheck TGEV Recombinant kit showed an excellent agreement with the TGEV-positive herd (76/90) compared to the other commercial kit (SVANOVIR® TGEV/PRCV-Ab) (50/90).

b. Stability

Six 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) post-production. 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.

Samples	Reactivity	Serial A				Serial B				Serial C			
		0 mo.	12 mo.	18 mo.	24 mo.	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	13,9	10,0	12,8	15,7
Sample 133	**Negative**	0,4	1,5	12,1	1,9	5,6	0,0	10,4	13,2	4,4	2,2	1,8	9,4
Sample 135	**Negative**	4,8	0,0	0,0	0,0	11,7	4,0	9,1	0,3	0,0	11,0	0,0	10,1
Sample 136	**Positive**	91,4	88,3	89,5	84,7	92,5	89,9	87,7	89,3	91,9	92,4	91,2	91,1
Sample 138	**Positive**	81,0	74,1	77,0	74,0	83,3	80,3	76,1	79,5	80,8	80,0	78,1	79,1
Sample 228	**Positive**	86,5	83,4	83,3	84,5	87,4	86,0	84,2	84,6	90,3	88,6	88,4	89,7

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

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c. Repeatability

The intra-plate (A), inter-plate (B) and inter-lot (C) repeatability was evaluated using 5 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

Sample	Well 1	Well 2	Well 3	Mean ± SD	CV (%)
Negative	1,494	1,327	1,301	1,374 ± 0,105	**7,6**
Negative	1,372	1,375	1,304	1,350 ± 0,040	**3,0**
Weak Positive	0,823	0,919	0,800	0,847 ± 0,063	**7,5**
Weak Positive	0,656	0,694	0,692	0,681 ± 0,021	**3,1**
Positive	0,189	0,207	0,239	0,212 ± 0,025	**12,0**

B

Sample	Well 1	Well 2	Well 3	Mean ± SD	CV (%)
Negative	1,494	1,450	1,428	1,457 ± 0,034	**2,3**
Negative	1,372	1,481	1,325	1,393 ± 0,080	**5,8**
Weak Positive	0,823	0,848	0,834	0,835 ± 0,013	**1,5**
Weak Positive	0,656	0,648	0,633	0,646 ± 0,012	**1,8**
Positive	0,189	0,193	0,222	0,201 ± 0,018	**8,9**

C

Sample	Serial A	Serial B	Serial C	Mean ± SD	CV (%)
Negative	1,494	1,413	1,273	1,393 ± 0,112	**8,0**
Negative	1,372	1,457	1,302	1,377 ± 0,078	**5,6**
Weak Positive	0,823	0,810	0,826	0,820 ± 0,009	**1,0**
Weak Positive	0,656	0,722	0,711	0,696 ± 0,035	**5,1**
Positive	0,189	0,233	0,172	0,198 ± 0,031	**15,9**

In both intra- and inter-plate studies, the optical density of all tested samples had a coefficient of variation lower than 15%. Only one sample had a CV greater than 15% (15.9) in the inter-lot study, but the SD of the sample was lower than most other samples. These data clearly demonstrate the high reproducibility of the Swinecheck TGEV Recombinant kit.

CONCLUSION

The Swinecheck® TGEV Recombinant 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.

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KIT COMPOSITION

Components	Quantity TRM-534	Quantity TRM-539
12 strips of 8 wells coated with recombinant protein S of TGEV	2	5
Ready-to-use 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 Conjugate (blue)	2 x 11 mL	55 mL
Concentrated Wash Solution (10X)	125 mL	125 mL
Ready-to-use Substrate	25 mL	60 mL
Ready-to-use Stop Solution	25 mL	60 mL

CFIA-licensed; 880DR/T5.0/D10

For more info, contact us at: 1-888-824-6838, option 3
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