Calculation of the Inhibition Percentage

Calculate the mean (M) of ODs obtained for the negative control serum (N). Divide the OD obtained for each sample by the MOD N. Then, subtract this result from 1 and multiply by 100 to obtain the inhibition percentage.

\[
\text{Inhibition percentage (\%) } = \left[ 1 - \frac{OD}{MOD} \right] \times 100
\]

Validity Criteria

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

| Mean OD positive control (MOD P) | < 0.3 |
| Mean OD negative control (MOD N) | > 0.9 |

Interpretation

- Sample inhibition % ≥ 55%: TGEV-Ab positive.
- Sample inhibition % < 45%: negative.
- Sample inhibition % ≥ 45% but < 55%: TGEV-Ab suspicious. A second serum sample should be collected 2 weeks later and retested.

BIBLIOGRAPHY


Transmissible Gastroenteritis Virus Antibody Test Kit, ELISA

Swinecheck® TGEV Recombinant

Insert

This kit is based on an immunoenzymatic assay for the detection of antibodies against transmissible gastroenteritis virus (TGEV) in porcine serum.

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.1-3

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 antigen3.

PRINCIPLE OF THE TEST

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 Mab binds to the protein sites that have not been bound by the serum Ab present in positive samples. After incubation, the excess of Mab is eliminated by a second wash and its 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 are read. The intensity of the color allows the determination of the type of sample tested. A strong positive will show a weak reaction (pale yellow), whereas a negative sample will show a strong reaction (dark yellow). All shades of yellow between dark and pale represent various degrees of positivity.
MATERIAL

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 strips of 8 wells coated with recombinant protein S of TGEV</td>
<td>2</td>
</tr>
<tr>
<td>Ready-to-use positive control</td>
<td>2 mL</td>
</tr>
<tr>
<td>Ready-to-use negative control</td>
<td>2.5 mL</td>
</tr>
<tr>
<td>Ready-to-use sample dilution buffer (green)</td>
<td>30 mL</td>
</tr>
<tr>
<td>Ready-to-use conjugate (blue)</td>
<td>2 X 11 mL</td>
</tr>
<tr>
<td>Concentrated wash solution (10X)*</td>
<td>125 mL</td>
</tr>
<tr>
<td>Ready-to-use substrate</td>
<td>25 mL</td>
</tr>
<tr>
<td>Ready-to-use stop solution</td>
<td>25 mL</td>
</tr>
</tbody>
</table>

* Crystals may form when wash solution is kept at 2-7°C. This will not affect the efficiency of the product. In order to use this solution, simply bring it to room temperature and the crystals will dissolve.

The materials provided are sufficient for testing up to 184 samples in one test by distributing the samples on the 2 microplates and distributing the appropriate controls on each microplate.

The materials required but not provided:
- Purified water
- Adjustable single- and multi-channel micropipettes
- Single-use micropipette tips
- ELISA microplate washer (optional)
- Test tubes for sample dilution
- ELISA 96-well microplate reader equipped with 450 nm filter
- Containers for dilution of other solutions

PROCEDURE

A. Sample preparation

Dilute porcine serum samples 1/2 in sample dilution buffer (e.g., 200 µL sample in 200 µL 1X sample dilution buffer). It is important to use a new tip for each sample. Make sure each dilution is properly mixed before being distributed into the wells.

B. Preparation of wash solution

After homogenizing the concentrated wash solution (no evidence of crystals), dilute at 1/10 with purified water (e.g., 100 mL 10X concentrated wash solution in 900 mL purified water for each plate). Once diluted, the solution (1X) is stable for 1 week at 2-7°C.

C. Washings

THOROUGH WASHINGS ARE CRITICAL TO GET SUITABLE RESULTS. It is strongly recommended to use a plate washer or a pipette (do not use a wash bottle). It is important to drain all liquid contained in the plate after each wash and, after the last wash, to dry the plate by tapping it on absorbent paper. Be careful not to mix wells’ contents.

D. Test procedures

Bring all reagents to room temperature and mix well manually before use. Unused conjugates have to be stored back at 2-7°C as soon as possible.

1. Make a schematic representation of the distribution of controls and samples on the plate.
2. Dispense 100 µL of ready-to-use positive control, ready-to-use negative control or diluted samples (see section A) into appropriate wells (it is recommended to run control sera in duplicates).
3. Cover the wells and incubate at 37 ± 1°C for 1 hour.
4. Wash each well 3 times with 300 µL 1X wash solution (see sections B and C).
5. Dispense 100 µL of ready-to-use conjugate into each well.
6. Cover the wells and incubate at 37 ± 1°C for 30 minutes.
7. Wash each well 6 times with 300 µL 1X wash solution (see sections B and C).
8. Dispense 100 µL of ready-to-use substrate into each well.
9. Cover the wells and incubate, away from light, at 23 ± 2°C for 10 minutes.
10. Dispense 100 µL of ready-to-use stop solution into each well.
11. Measure optical densities (OD) at 450 nm. The reading should be done no later than 15 minutes after the addition of the stop solution.
12. Calculate the results.