Bovine Viral Diarrhea/Mucosal Disease: Mixed Antigen Screening ELISA: VF-P00623

Introduction

The bovine viral diarrhoea virus (BVDV) is a pestivirus, which causes two diseases: Bovine Viral Diarrhoea (BVD) and Mucosal Disease (MD).

Bovine Viral Diarrhoea is a pathology induced by one of the two strains of the virus (cytopathogenic and non-cytopathogenic). The acute form, characterised by fever and diarrhoea, is transient, with high morbidity rates and low mortality rates. Adult animals can also be infected with an asymptomatic subclinical form. Mucosal Disease has low morbidity rates (1%), but high mortality rates. More often it is characterised by ulcers at different levels of the digestive tract and diarrhoea that is often haemorrhagic.

Many pathologies are associated with or aggravated by the BVDV, including, among others, respiratory diseases, slow development, congenital defects, etc. The BVDV infection causes large annual economic losses to the European cattle industry, and has a negative impact on animal welfare.

When to Test

This kit detects the highly preserved NSP 2-3 (P80/125) protein of BVDV and the the E0 (E™/gp48) protein of BVDV in serum, whole blood, plasma, leucocyte extracts, organ extracts and blood clots. It is based on an "ELISA sandwich" technique. The test should be carried out at the first signs of a case of diarrhoea in order to determine the cause.

Sampling Recommendations

Follow veterinary advice in line with a case of diarrhoea.

Assay Description

Microtitre plates are supplied pre-coated with antibodies against NSP2-3 and E0. The sample to be tested is incubated with a buffer containing a polyclonal antibody specific to BVDV. If BVDV is present in the sample then a polyclonal antibody-antigen-monoclonal antibody complex is formed. Unbound material is washed from the wells. An antibody conjugated to peroxidase, specific to the polyclonal added in the first stage is added. In presence of the BVDV in the sample, this conjugate binds on the complex formed in the well of the plate. The conjugate is washed form the plate and a TMB substrate is added, the degree of colour intensity (optical density) is directly related to the amount of antigen present in the sample.

Assay Procedure

For further information or to place an order please contact us at:
Guildhay Limited, 6 Riverside Business Centre, Walnut Tree Close, Guildford, Surrey, GU1 4UG England
Tel: +44 (0)1483 573727 Fax: +44 (0)1483 574828
Email: sales@guildhay.co.uk Website: http://www.guildhay.co.uk
Interpretation of Results

For interpretation of the results a SP % value must be calculated as follows:

\[ S/P \% = \frac{\text{SAMPLE ABSORBANCE} - \text{NEGATIVE CONTROL ABSORBANCE}}{\text{POSITIVE CONTROL} - \text{NEGATIVE CONTROL}} \times 100 \]

RESULT INTERPRETATION FOR SAMPLES

<table>
<thead>
<tr>
<th>S/P % Value</th>
<th>BVDV P80 status</th>
</tr>
</thead>
<tbody>
<tr>
<td>\leq 25</td>
<td>Negative</td>
</tr>
<tr>
<td>25-30</td>
<td>Doubtful</td>
</tr>
<tr>
<td>\geq 30</td>
<td>Positive</td>
</tr>
</tbody>
</table>
ELISA DETECTION OF NSP2-3 AND E0 OF THE BOVINE VIRAL DIARRHOEA/MUCOSAL DISEASE VIRUS (BVDV) IN SERUM, WHOLE BLOOD, PLASMA, LEUCOCYTES EXTRACTS AND BLOOD CLOTS.

(192 REACTIONS)
INTRODUCTION

The bovine viral diarrhoea virus (BVDV) is a pestivirus, which causes two diseases: Bovine Viral Diarrhoea (BVD) and Mucosal Disease (MD).

Bovine Viral Diarrhoea is a pathology induced by one of the two strains of the virus (cytopathogenic and non-cytopathogenic). The acute form, characterised by fever and diarrhoea, is transient, with high morbidity rates and low mortality rates. Adult animals can also be infected with an asymptomatic subclinical form.

Mucosal Disease has low morbidity rates (1%), but high mortality rates. More often it is characterised by seromucoid nasal secretions, ulcers at different levels of the digestive tract, diarrhoea that is often haemorrhagic.

Many pathologies are associated with or aggravated by the BVDV, including, among others, respiratory diseases, slow development, congenital defects, etc.

Mucosal Disease occurs in calves infected during gestation (Immunotolerant Persistently Infected (I.P.I.) animals). These animals were infected with a non-cytopathogenic strain by the transplacental way between the 42nd and the 120th day of gestation. This corresponds to a period, when immunocompetence is being established in the foetus: foreign antigens present at this time are considered as self-antigens and no immune response is developed against them. Thus, the persistently infected animals do not produce antibodies against the strain they are infected with. Mucosal Disease is induced by superinfection with a cytopathogenic strain antigenically similar to the non-cytopathogenic strain present in the animal.

The main sources of infection are I.P.I. animals that continuously produce and shed the virus, and, in a transient manner (during 10 days), animals recently contaminated with a primary BVDV infection. The transmission of the virus can be oral-nasal, conjunctival, genital or transplacental.

The detection of virus antigens is used to detect the presence of IPI in livestock. Historically based on virus isolation on cell culture, this antigenic detection is currently performed by ELISA technique. This method is easy to implement, rapid, reliable and is particularly suited to the analysis of a large number of samples.

This kit detects NSP 2-3 (P80/125) et E0 (=Ems or gP48) of BVDV in serum, whole blood, plasma, leucocytes extracts, organs extracts and blood clots.

PRINCIPLE OF THE TEST

The principle of the test is:

1) Specific monoclonal antibodies directed to NSP2-3 and E0 are supplied coated on all the microplate wells.

2) The samples to be tested are co-incubated in the wells with a buffer containing a polyclonal antibody directed specifically to BVDV. If the BVDV antigens are present in the analysed sample, a polyclonal antibody-antigen-monoclonal antibody complex is formed.

3) After washing, a Peroxidase conjugate directed to the polyclonal antibody is incubated in the wells. In presence of the BVDV antigens NSP 2-3 or E0 in the sample, this conjugate binds on the plate.

4) After another washing step, the enzyme substrate (TMB) is added to the conjugate, forming a blue compound becoming yellow after blocking. The intensity of the colour is a measure of the BVDV level in the sample.

The cut-off is set by using a positive control, which must be added to each microplate.
### KIT CONTENTS and STORAGE OF REAGENTS

It is recommended to bring at room temperature (21°C ± 5°C) all the reagents of the kit at least one hour before use (except the conjugate and the controls).

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>QUANTITY</th>
<th>STORAGE AND NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monowell coated microplates</td>
<td>2</td>
<td>+5°C (± 3°C) • Divisible microplates 1 x 16 • If a microplate is not entirely used, it may be stored for later use if it is immediately closed in an airtight container and stored at +5°C (± 3°C).</td>
</tr>
<tr>
<td>Concentrated (20x) wash solution</td>
<td>1 x 100 ml bottle</td>
<td>+5°C (± 3°C) • May form crystals at +5°C (± 3°C), which rapidly disappear at +21°C (± 5°C). A gentle shaking of the solution will accelerate the dissolution of the crystals • This solution can also be stored at +21°C (± 5°C) for 1 month, if the vials are closed in an airtight way, in order to be immediately ready to use when needed. • The “Concentrated (20x) wash solution” is the same for all the kits of the INSTITUT POURQUIER and can be used equally in the different kits. • After dilution, the Wash Solution can be stored for 3 days at +5°C (± 3°C)</td>
</tr>
<tr>
<td>Haemolysis Buffer (5x) pink</td>
<td>1 x 120 ml bottle</td>
<td>+5°C (± 3°C) • After dilution, the Haemolysis Buffer can be stored at +5°C (± 3°C) up to 1 month.</td>
</tr>
<tr>
<td>Dilution Buffer 9 blue</td>
<td>1 x 6 ml bottle</td>
<td>+5°C (± 3°C) Shake before using</td>
</tr>
<tr>
<td>Dilution Buffer 23 light green</td>
<td>1 x 50 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td>Positive Control (liquid)</td>
<td>1 x 0,4 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td>Negative Control (liquid)</td>
<td>1 x 0,4 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td>Polyclonal anti – E0 / NSP2/3 antibody dark red</td>
<td>1 x 1,5 ml bottle</td>
<td>+5°C (±3°C)</td>
</tr>
<tr>
<td>Anti-polyclonal antibody conjugate yellow</td>
<td>1 x 2,5 ml bottle</td>
<td>+5°C (±3°C) • The diluted conjugate cannot be stocked.</td>
</tr>
<tr>
<td>Revelation solution 5 (TMB) Ready to use</td>
<td>1 x 30 ml bottle</td>
<td>+5°C (± 3°C) • This solution can be left on the draining board at 21°C (± 5°C) up to 1 week (if the vial is well closed in an airtight way), in order to be immediately available when needed.</td>
</tr>
<tr>
<td>Stop Solution (H₂SO₄ 0,5M solution)</td>
<td>1 x 60 ml bottle</td>
<td>+5°C (± 3°C) • It can be stored at +21°C (± 5°C) for 1 month (if the vial is well closed in an airtight way), in order to be immediately ready to use when needed. • The “Stop solution” is the same for all the kits of the INSTITUT POURQUIER and can be used equally in the different kits</td>
</tr>
</tbody>
</table>

**Using Instructions**
MATERIALS REQUIRED BUT NOT INCLUDED IN THE KIT

1) Microplates reader
2) Centrifuge
3) Centrifuge tubes and microtubes
4) Vortex or similar type of agitator
5) Haemolysis microtubes of 5 ml
6) Scissors or Scalpel
7) Syringe of 10 ml
8) Triton-X-100 (a SIGMA product ref X 100)
9) Microplate washing system that distributes 300 µl per well
10) Precision Micropipettes and multi-dispensing micropipettes
   (The precision required must be lower or equivalent to 5% of the volumes indicated)
11) Disposable pipette tips
12) Distilled water: The water used for the reconstitution of the Haemolysis Buffer and of the Concentrated wash solution may be produced by a conventional distillation system or any other high-performance water purification system (reverse osmosis, resin or activated charcoal purification ...).
13) Microplate covers

PRECAUTIONS FOR USE

1) Do not place the pipette in the mouth when testing reagents.
2) Avoid contact of the substrate (TMB*) with skin, mucous membranes, and eyes.
3) "Stop-solution" contains H2SO4*(0,5M) acid, that can cause serious burns in case of contact with skin, mucous membranes and eyes.
4) Although the material delivered in the package does not contain any contaminating element, and that bovine blood samples are, in theory, non-infectious, it is nonetheless advised to decontaminate the whole disposable elements used by immersion for at least 1 hour in freshly prepared 5% sodium hypochlorite, before eliminating them, or autoclaving them at 120°C for a minimum of 1 hour.

* The toxicity form of the products is available at the Institut Pourquier.

INSTRUCTIONS FOR USE

1) PRELIMINARY STAGES

a) Preparation of sample incubation buffer
   - The sample incubation buffer is prepared by dilution at 1/10 of the polyclonal anti–E0 / NSP2/3 antibody in the dilution buffer 23.

b) Preparation of Leukocyte Extract
   - Dilute a bottle of "Concentrated Haemolysis Buffer (5X) " in 480 ml of distilled water. This solution is hereafter called "Haemolysis Buffer".
   - Dispense 2 ml of whole blood in a centrifuge tube
   - Add 3 ml of « Haemolysis Buffer »
   - Shake with a Vortex
   - Incubate for 10 to 15 minutes at room temperature (+21°C ± 5°C) until the complete lysis of the red blood cells (after shaking the liquid on the surface of the tube must be translucent)
   - Centrifuge for 15 minutes at 1000 g
   - Eliminate the supernatant, and drum the tube turned upside down on an absorbent-paper.
   - Dilute the pellet of leucocytes with 200 µl of « Dilution Buffer 9 » and mix with a pipette by aspirating and reversing 5 to 6 times [see note 3].
   - Leave to settle few minutes; lumps can appear with a more liquid supernatant phase. In that case, take this liquid phase for the test.
Notes:
1. It is also possible to work on higher or lower volumes of whole blood samples, if the proportions are respected.
2. The leukocyte extract keeps a red colour due to haemoglobin traces. Our experience has shown that the presence of haemoglobin does not interfere on the quality of the results. Nevertheless the haemoglobin traces can be eliminated by one or two washing cycles with Haemolysis Buffer.
3. The quantity of dilution buffer delivered in the kit is sufficient for 30 leukocyte extractions. Extra reagents can be supplied free of charge, on request.

b) Preparation of organ extracts (spleen, lung)
- Remove a sample of 0.5 cm³ of organ
- Cut in small pieces with a scissors or scalpel in a haemolysis tube
- Add 1ml of « Dilution Buffer »
- Homogenize by shaking
- Incubate for 30 minutes at +21°C (± 5°C), preferentially under shaking
- Centrifuge for 15 minutes at 1000 g
- Collect the supernatant (generally situated just under the organ to eliminate)

c) Preparation of blood clots
- Remove half of a clot of 10 ml blood
- Dispense the clot with a syringe of 10 ml in a centrifuge tube
- Add 2 ml of wash solution containing 1% of Triton-x-100 (see “material required, but not included in the kit”)
- Shake and incubate for 30 minutes at +21°C (± 5°C)

2) DEPOSITING THE SAMPLES
- Controls and samples (whole blood, serum, plasma, leucocytes, clot and organ extracts) are diluted to ½ as follows:
  - Dispense:
    - 50 µl of sample incubation buffer per well
    - 50 µl of undiluted negative control in A1
    - 50 µl of undiluted positive control in B1 and C1 (see: notes 2 and 3)
    - 50 µl of sample to be tested per well
- Homogenize the content of the wells by gently shaking the plate (see note 1).
- Cover the plate (with a cover, aluminium or adhesive foil)
- Incubate for 90 minutes (± 5 min.) at +21°C (±5°C)

<table>
<thead>
<tr>
<th>A</th>
<th>N</th>
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<tbody>
<tr>
<td>B</td>
<td>P</td>
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<td>C</td>
<td>P</td>
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<td>D</td>
<td>1</td>
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<td>2</td>
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<td>4</td>
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<tr>
<td>H</td>
<td>5</td>
</tr>
</tbody>
</table>

Notes:
1. The individual filling of the 96 wells is sometimes a long process. In order to standardise the sera incubation time, the controls and serum samples are prepared in plates with 96-U-shaped-bottom-wells. It is therefore possible to transfer them rapidly (column by column) by using a multi-channel pipette.
2. The position of controls in A1, B1, and C1 is not important, they may be dispensed anywhere on the plate.
3. Sometimes, laboratories, which use automatic methods, may not have enough reagents (i.e. “concentrated (20x) wash solution” or “Dilution buffer 23”...). Extra reagents can be supplied free of charge, on request.
3) WASHING

a) Dilute a vial of "concentrated (X 20) wash solution" in 1900 ml of distilled water. This solution is hereafter called "wash solution". The dilution can be carried out before the elimination of the crystals which appeared at +5°C (± 3°C) on condition that the whole 100 ml vial is used.

b) Empty the content of the plate by «flick-off» or better by an automatic method.

c) Fill all the wells of the plate with the wash solution; then empty them again.

d) Repeat the step c) twice (a total of 3 washes).

Notes:
1. According to the equipment used for the washing step, the number of washings can be increased or reduced. A good washing has to lead to the removal of all the red traces of blood in the wells.
2. When several plates are processed at the same time, it is possible (in order to synchronize all the steps) to leave the plates full of "Wash solution" up to one hour without modifying the validity of the test.

4) DEPOSITING THE CONJUGATE

a) Dilute the conjugate to 1/10 in wash solution

b) Dispense 100 µl of conjugate per well.

c) Cover the plate (with a cover, aluminium foil or adhesive) and incubate for 30 minutes (± 3 min.) at +21°C (±5°C).

5) WASHING

a) Empty the content of the plate by «flick-off» or by an automatic method.

b) Fill all the wells on the plate with the wash solution; then empty them again.

c) Repeat the step b) three times (total of 4 washes).

Note:
1. Particular care with the last wash is very important in getting a good test result.
2. If the wash is carried out with a manual method, it is possible after the last wash to drum the microplate on a dry towel in order to empty the wells completely.

6) REVELATION

a) Dispense 100 µl of "Revelation Solution 5" ready to use per well

b) Incubate the plate for 20 minutes at +21°C (± 5°C) (away from the light).

c) Stop the reaction by adding 100 µl of "stop solution" per well.

d) Shake gently the plate until the coloured solution is homogenised. Wipe carefully the underside of the plate.

Note:
1. The 20-minute-revelation-period, which is indicated in the method, gives the O.D. values provided in the paragraph "INTERPRETATION" when performed in our laboratories. However the rate of colour revelation can be slightly modified by different factors (quality of the washes, quality of the water used, precision of the pipetting, temperature of the reaction...). So, the user may stop the reaction at 20 minutes ± 10 minutes.
2. The reading can be done up to 1 hour after blocking if the microplates are stored away from the light.

7) READING

Read the optical densities at 450 nm (OD.450). The photometer must first be blanked on air.
VALIDATION CRITERIA

The results can be considered reliable if:

- The mean OD of the positive control reaches at least a value of 0.800.
  and
- A ratio between the mean OD 450 value of the positive control and the OD 450 value of the negative control is greater than or equal to 4.5

INTERPRETATION

Calculate for each sample the S/P ratio (in %):

\[
S/P \% = \frac{(\text{OD of the sample} - \text{OD of the negative control})}{(\text{mean OD of the positive control} - \text{OD of the negative control})} \times 100
\]

- Samples with a S/P% equal to or lower than 25% are considered to be from animals that are not carriers of the BVDV.
- Samples with a S/P% between 25% and 30% are considered to be doubtful.
- Samples with a S/P% equal to or greater than 30% are considered being from animals that are carriers of the BVDV (IPI).

Notes:
A sample, which gives a doubtful result, may come from a transiently infected adult animal. It is thus recommended to confirm doubtful results by antigenic and serological analyses a few weeks later.

SUMMARY

<table>
<thead>
<tr>
<th>S/P %</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 25%</td>
<td>Negative</td>
</tr>
<tr>
<td>25% &lt; S/P% &lt; 30%</td>
<td>Doubtful</td>
</tr>
<tr>
<td>≥ 30%</td>
<td>Positive</td>
</tr>
</tbody>
</table>