SEROLOGICAL DETECTION OF IGM ANTIBODIES
DIRECTED TO THE BOVINE RESPIRATORY SYNCYTIAL VIRUS
BY ELISA IN SERUM
96 Reactions
INTRODUCTION

The bovine respiratory syncytial virus (BRSV) is a pneumovirus responsible for severe respiratory infections in young cattle and dairy herds. The transmission is horizontal by nasal secretions. It causes an infection of the lower respiratory tract with after-effects and important economic losses. The clinical signs are non-specific: pneumonia accompanied by hyperthermia and polypnoea. Laboratory analyses are therefore necessary for the identification of the causative agent.

This diagnostic tool allows detection of IgM antibodies specifically directed to the BRSV in individual sera. The presence of IgM antibodies is a sign for a recent contact of the animal with the BRSV (<15 days post-infection). This kit can give information about a recent infection by analysing only one sample of the animal. It is not necessary to follow up the IgG seroconversion using two consecutive samples. Furthermore, colostrum antibodies do not influence the IgM result. This ELISA can be used for calves less than 12 months. After this age the possible presence of rheumatoid factors can interfere with the reaction leading to false positive results.

PRINCIPLE OF THE TEST

1. The BRSV antigen is supplied coated to the wells of the polystyrene microplate (only the even-numbered columns are specifically coated).
2. The sera to analyse are diluted and incubated in the wells of the microplates. If BRSV-specific IgM antibodies are present in the sample, they form antigen-antibody complexes, by which the antibodies are fixed to the microplate wells.
3. After washing, a monoclonal anti-bovine IgM antibody coupled to an enzyme (Horse Radish Peroxidase) is added in the wells and incubated. This conjugate binds to the immune-complex.
4. After washing, the enzyme substrate (TMB) is added to the wells. If the enzyme is fixed in the wells, it transforms the substrate into a blue compound becoming yellow after blocking. The intensity of the colour is a measure of the antibody level in the sample.

The cut-off is set by using a control supplied with the kit (¢positive control'), which must be added to each plate.

MATERIAL REQUIRED BUT NOT INCLUDED IN THE KIT

1. Microplate spectrophotometer
2. Centrifuge
3. Centrifuge tubes and microtubes
4. Vortex or similar type agitator
5. Microplate washing system that distributes 300 µl per well
6. Precision micropipettes and multi-dispensing micropipettes (the precision required must be ≤10% for volumes ≤10 µl and ≤5% for the other indicated volumes)
7. Disposable pipette tips
8. Distilled water: the water used for the dilution of the «Concentrated (20x) wash solution» may be produced by a conventional distillation system or any other high-performance water purification system (reverse osmosis associated to ionic exchange resins and activated charcoal purification ...).
9. Microplate covers (aluminium or adhesive foil)
**KIT CONTENTS AND STORAGE OF REAGENTS**

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

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>QUANTITY</th>
<th>STORAGE AND NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coated microplates</td>
<td>2</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If a microplate is not entirely used, it may be stored for later use during 3 months if it is immediately closed in an airtight container and stored at +5°C (± 3°C).</td>
</tr>
<tr>
<td>Concentrated (x 20) wash solution</td>
<td>1 x 100 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• May form crystals at +5°C (± 3°C), which rapidly disappear at +21°C (± 5°C). A gentle agitation of the solution will accelerate the dissolution of the crystals.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• This solution can also be stored at +21°C (± 5°C) for 1 month, if the vials are well closed, in order to be immediately ready to use when needed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• The “Concentrated (x 20) wash solution” is the same for all the kits of INSTITUT POURQUIER and can be used equally in the different kits.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• After dilution, the Wash Solution can be stored for 3 days at +5°C (± 3°C)</td>
</tr>
<tr>
<td>Dilution Buffer 2 green (for samples)</td>
<td>1 x 120 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td>Dilution Buffer 1 blue (for conjugate)</td>
<td>1 x 120 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td>Positive control liquid</td>
<td>1 x 0.5 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td>Negative control liquid</td>
<td>1 x 0.5 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td>Monoclonal conjugate anti-bovine IgM / peroxidase</td>
<td>1 x 0.3 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• The diluted conjugate solution cannot be stored</td>
</tr>
<tr>
<td>Revelation solution 2 Ready to use (TMB)</td>
<td>1 x 30 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• This solution can be slightly bluish at +5°C (± 3°C) and becomes colourless at +21°C (± 5°C).</td>
</tr>
<tr>
<td>Stop Solution (H₂SO₄ 0.5M solution)</td>
<td>1 x 120 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• It can be stored at +21°C (± 5°C) up to 1 month (if the vial is well closed), in order to be immediately available when needed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• It is the same for all the kits of INSTITUT POURQUIER and can be used equally in the different kits.</td>
</tr>
</tbody>
</table>

Using instructions
**PRECAUTIONS FOR USE**

1. Do not place the pipette in the mouth when testing reagents.
2. «Stop-solution» containing H\textsubscript{2}SO\textsubscript{4}(0.5M) acid, could cause serious burns in case of contact with skin, mucous membranes and eyes.
3. Even if the material delivered in the package does not contain any contaminating element, and that the sera are, in theory, non-infectious, it is nevertheless recommended to decontaminate the whole disposable elements used during the course of handling by using immersion for a minimum of 1 hour in freshly prepared 5% sodium hypochlorite, prior to eliminating them, or to use the autoclave at 120°C for a minimum of 1 hour.

*The product’s toxicity data sheet is available at the Institut Pourquier.*

**INSTRUCTIONS FOR USE**

1) **DEPOSITING THE SAMPLES**

Sera (controls and samples) are diluted to 1/20 and incubated for 1 hour (± 5 min.) at 21°C (± 5°C) as described below:

- **Dispense** (following figure 1)
  - 190 µl of “Dilution buffer 2” per well.
  - 10 µl of undiluted negative control in A\textsubscript{1} and A\textsubscript{2}
  - 10 µl of undiluted positive control in B\textsubscript{1} / B\textsubscript{2} and C\textsubscript{1} / C\textsubscript{2}
  - 10 µl of each undiluted sample to be tested in:
    - 1 uncoated well (odd-numbered column)
    - 1 coated well (even-numbered column)

- **Homogenize** the contents of the wells by gently shaking the plate (see note 1).
- **Cover** the plate (with a lid, aluminium foil or adhesive) and leave to incubate for 1 hour (± 5 min) at 21°C (± 5°C).

This method enables the test to be implemented in less than half a day.

![Figure 1: Distribution of the Samples](image)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>N</th>
<th>6</th>
<th>6</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>P</td>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>P</td>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**N** = Negative control
**P** = Positive control

1 = Sample to be tested n° 1
2 = Sample to be tested n° 2
3 = .................................
Note:
1. The individual filling of the 96 wells is sometimes a long process. In order to standardize the sample incubation time, the controls and samples are prepared in plates with 96 wells with U-shaped-bottoms. It is therefore possible to transfer them rapidly (column by column) using a multi-dispensing pipette. It is nevertheless essential to dilute the samples in the same way as the controls.
2. The position of the control in A1/A2, B1/B2 and C1/C2 is not very important. They may be dispensed anywhere on the plate.
3. Laboratories, which use automatic methods or which prefer pre-diluting the samples in order to improve the reproducibility, may not have enough reagents (ie "concentrated x 20 wash solution" or "Dilution Buffer "). Extra reagents can be supplied free of charge, on request.

2) 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 crystals appeared at +5°C (±3°C), so long as the whole 100 ml vial is used.
b) Empty the content of the plate by a "flick-off" method or better by a manual or 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).

Note:
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" during one hour without modifying the validity of the test.

3) DEPOSITING THE CONJUGATE
a) Dilute the conjugate to 1/100 in "Dilution Buffer 1".
b) Dispense 100 µl of this diluted 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).

4) WASHING
a) Empty the content of the plate by turning it over or by another manual or automatic method.
b) Empty the content of the plate by a "flick-off" method or better by a manual or automatic method.
c) Repeat the step b) twice (total of 3 washes)

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

5) REVELATION
a) Dispense 100 µl of "Revelation solution No 2" per well.
b) Incubate the plate at +21° (± 5°C) for 20 minutes (away from the light).
c) Dispense 100 µl of "stop solution" per well.
d) Shake gently the plate until the coloured solution is homogenized. Wipe carefully the underside of the plate.
Note:
1. The 20 minutes revelation period as outlined above gives O.D. values conform to values provided in the paragraph "INTERPRETATION", when performed in our laboratories. Nevertheless the rate of colour revelation can be slightly modified by different factors (quality of the washes, quality of water used, precision of the pipetting, temperature of the reaction...). Depending then on these factors, the revelation step may give O.D. values higher or lower than those expected. In this case, the reaction may be stopped after 20 minutes ±10 minutes.
2. The reading can be done up to 1 hour after having stopped the reaction, if the plates are kept in the dark.

6) READING
a) Read the optical densities at 450 nm (OD.450). The photometer must be blanked on air.
b) Calculate the corrected OD.450 for each sample: subtract the OD.450 value obtained from the uncoated well from the OD.450 from the coated well.

VALIDATION CRITERIA
The results can be considered reliable if:

The mean uncorrected OD 450 value of the positive control is ≥ 0.350 and
A ratio between the corrected OD 450 value of the positive control and corrected OD 450 value of the negative control is greater than or equal to 3.5.

Note:
The corrected OD 450 value of the negative control can be found «negative» or at zero. In that case, use the absolute value for the validation.

INTERPRETATION
Calculate for each sample, the ratio S/P:

\[ \text{Sw/P} = 100 \times \frac{\text{Corrected OD 450 value of the Sample}}{\text{Mean corrected OD 450 value of the Positive Control}} \]

- Any sample with a S/P ≤ 20% is considered to be from an animal, which had not recently been in contact with the BRSV (<15 days post-infection).
- Any sample with a S/P > 20% is considered to be from an animal, which had recently been in contact with the BRSV (<15 days post-infection).

LEGEND

= Modification in the using instructions
= Minor modification in the text