Introduction

Bovine herpesvirus–1 (BHV-1) is divided into two sub-classes these are BHV-1.1 and BHV-1.2. The BHV-1.1 virus is the causative agent of Infectious Bovine Rhinotracheitis (IBR) which is a highly infectious respiratory disease. The BHV-1.2 virus is associated with infectious pustular vulvovaginitis (IPV) a venereal disease of cattle, which is further dived into abortogenic and non-abortogenic sub-groups. BHV-1 manifests itself in various ages and types of cattle with other syndromes including encephalitis and systemic disease in newborn calves, in dairy cattle there can be a dramatic drop in milk production. Respiratory tract infections are usually confined to the upper respiratory tract (nose, throat, and wind-pipe). Signs include difficult inhalation rapid breathing; and a profuse, clear, watery nasal discharge that, as the infection progresses, becomes a sticky yellow discharge that hangs in long strands from the nostrils. Most infected animals rapidly lose weight, and eventually the nostrils become encrusted. If the crust on the nostrils is rubbed off, the nose looks very red and inflamed, hence the name "red nose." The IBR virus can persist in a clinically recovered animal for years laying dormant following an infection, it's thought to be re-activated by stresses. Animals infected with these "hidden" viruses are frequently considered to be reservoirs of the disease.

When to test

This kit detects the presence of all types of antibodies to the virus (envelope proteins, caspid proteins, etc ….) therefore the kit can be used to detect the presence of the virus at various stages of the infection. The disease tends to occur 10 – 20 days after the introduction of susceptible cattle and it can take less than a month for the disease to go through a pen of feedlot cattle in close confinement. Therefore, rapid sanitary measures and confirmation of the disease are required.

Sampling recommendations

Follow veterinary advice in line with a screening program or suspected outbreak.

Assay Description

Microtitire plates are supplied pre-coated with BHV-1 viral antigen. Diluted samples are incubated in the wells where any antibodies specific to BHV-1 binds and forms a complex. Unbound material is washed from the wells. A peroxidase conjugated immunoglobulin is added, this conjugate reagent binds to the immune-complex formed in the first stage of the test. Unbound conjugate is washed away and TMB substrate is added to the wells. The degree of colour intensity (optical density) is directly related to the amount of antibody present in the sample.

Assay Procedure

1. Add Sample/ Controls to the plate
2. Incubate 1 hour & Wash
3. Add Enzyme Conjugate
4. Incubate 30 mins & Wash
5. Add Substrate Reagent
6. Incubate 20 mins
7. Add Stop Sol. Read at 450nm
Interpretation of Results

For interpretation the verification sample absorbance is corrected against an uncoated well, for all results an SP % must be calculated as follows:

FOR SCREENING SAMPLES:

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

FOR VERIFICATION SAMPLES:

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

RESULT INTERPRETATION FOR THE SERUM SAMPLES:

<table>
<thead>
<tr>
<th>S/P % Value</th>
<th>BHV-1 Antibody status</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 45</td>
<td>Negative</td>
</tr>
<tr>
<td>45-55</td>
<td>Suspect</td>
</tr>
<tr>
<td>≥ 55</td>
<td>Positive</td>
</tr>
</tbody>
</table>

RESULT INTERPRETATION FOR THE MILK SAMPLES:

<table>
<thead>
<tr>
<th>S/P % Value</th>
<th>BHV-1 Antibody status</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 25</td>
<td>Negative</td>
</tr>
<tr>
<td>≥ 25</td>
<td>Positive</td>
</tr>
</tbody>
</table>

IBR / IPV test range:
VF-P03130 Serum & Milk Verification ELISA
VF-P03140 Serum & Milk Screening ELISA
VF-P03145 IBR / IPV: gB Serum Screening ELISA
SEROLOGICAL DIAGNOSIS OF
INFECTION BOVINE RHINOTRACHEITIS
BY ELISA METHOD

(Serum and Milk)

(960 Reactions)

VF-P03140-960 960test

VF-P03140-480 480test

ELISA IBR Serum and Milk Screening
Version: P03140/02 - 11/07/05
INTRODUCTION

IBR/IPV is a contagious disease caused by a type I Herpes virus. A respiratory syndrome associating cough, runny nose, elevated body temperature and bronchopulmonary complications are its main symptoms. More rarely, it can present ocular, nervous or genital forms (bovine pustular vulvo-vaginitis, IPV). It can also result in abortion and neonatal mortality.

As with all herpes viridae, BHV-1 can, after a primary infection, remain latent in the infected animal and may recur during later periods of viral reactivation. Vaccination is used to limit the spread of the virus within an infected herd, but it does not offer absolute protection: indeed, in areas which are widely infected or in farms where the virus is present, vaccination decreases or even eliminates the economic effects of the disease, it does not prevent the persistent spread of BHV-1 and its eventual reappearance.

That is why, as in the case of Aujesky’s Disease, sanitary programs are mainly based on serological screening and progressive elimination of seropositive animals (in areas of slight or medium prevalence).

Historically based on the seroneutralization or on passive serum agglutination method, this screening is now essentially done by ELISA. This method is easy to implement, rapid and reliable and is particularly well suited to the analysis of a large number of samples.

This kit uses an ultrapurified BHV-1 lysate and can be used to demonstrate the presence of all types of antibodies to the virus (envelope proteins, capsid proteins, etc...) in serum and milk samples.

Note: a focused research of specific anti-gB antibodies can be implemented on serum with a competition ELISA (contact the Institut Pourquier for the product codes and batch numbers).

PRINCIPLE OF THE TEST

The principle of the test is:
1) All the wells of the microplates are coated with BHV-1 viral antigen.
2) Samples to be tested are diluted and incubated in the wells. Any antibody specific to BHV-1 present in the serum will form a BHV-1 antibody immune-complex and remains bound in the wells.
3) After washing, an anti-bovine antibody Immunoglobulin coupled to an enzyme is added to incubate. This conjugate will bind to the immune-complex.
4) After another washing, the enzyme substrate (TMB) is added to the wells. If the conjugate is fixed in the wells, the enzyme will transform the substrate into a blue compound becoming yellow after blocking. The intensity of the colour is a measure of the rate of antibodies present in the sample to be tested.

The limit of positivity is defined by using a control serum supplied with the kit ("positive control"), which has to be added to each microplate.

DETECTABILITY

The kit is adapted for the analysis of individual sera and milk samples (bulk milk). The detectability limit of the kit allows the detection of the European standard serum EU2.
**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>10</td>
<td>+5°C (±3°C)</td>
</tr>
</tbody>
</table>
| • If a microplate is not entirely used, it may be stored for later use if it is immediately closed in an airtight way and stored at +5°C (±3°C).  
• The packaging of the plates leads to the appearance of deposits or crystals at the bottom of the wells, without modifying the reaction. |
| Concentrated (20x) wash solution | 2 x 100 ml bottles | +5°C (±3°C) |
| • May form crystals at +5°C (±3°C), but these 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) up to 1 month, if the vials are closed in an airtight manner, in order to be immediately ready to use when needed.  
• The "Concentrated (X 20) wash solution" is similar 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) |
| Dilution Buffer light green (for samples) | 2 x 120 ml bottles | +5°C (±3°C) |
| Dilution Buffer light blue (for conjugate) | 1 x 120 ml bottle | +5°C (±3°C) |
| Positive control | 1 x 1.0 ml bottle | +5°C (±3°C) |
| Negative control | 1 x 1.0 ml bottle | +5°C (±3°C) |
| Anti-bovine IgG-Peroxidase Conjugate | 1 x 1.5 ml bottle | +5°C (±3°C) |
| • The diluted conjugate solution cannot be stored |
| Revelation solution (TMB) Ready to use | 1 x 120 ml bottle | +5°C (±3°C) |
| • This solution can be slightly bluish at +5°C (±3°C) and becomes colourless at +21°C (±5°C).  
• Thus it 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 manner), in order to be immediately ready to use when needed. |
| Stop Solution (H₂SO₄ 0.5M solution) | 1 x 120 ml bottle | +5°C (±3°C) |
| • It can be stored at +21°C (± 5°C) up to 1 month (if the vial is well closed in an airtight manner), in order to be immediately ready to use when needed.  
• The "Stop solution" is similar for all the kits of the INSTITUT POURQUIER and can be used equally in the different kits. |
| Using Instructions |   |   |
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» which contains H₂SO₄*(0.5M) acid, can cause serious burns in case of contact with skin, mucous membranes and eyes.
4. Even if the material delivered in the package does not contain any contaminating element, and that the samples are, in theory, non-infectious, it is nevertheless 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 or by any other method in accordance with the regulation in force.

*M The Institut Pourquier is at your disposal to supply the toxicity data sheets of the product.

MATERIALS REQUIRED BUT NOT INCLUDED IN THE KIT

1. Microplates reader
2. Centrifuge
3. Tubes for centrifugation
4. Vortex or similar
5. Microplate washing system that distributes 300 µl per well
6. Precision Micropipettes and Multi-dispensing micropipettes (The precision required must be lower than or equivalent to 10% for volumes lower or equal to 10 µl and to 5% for all the other volumes indicated).
7. Disposable pipette tips
8. Distilled water: the water used for the reconstitution of the wash solution can be produced by a conventional distillation system or any other high-performance water purification system (reverse osmosis, resin or activated charcoal purification ...).
9. Microplate covers (lid, aluminium foil or adhesive)
10.Incubator at +37°C (±3°C)

USING INSTRUCTIONS FOR SERUM SAMPLES

1) DEPOSITING THE SAMPLES

Dilute the sera and controls to 1/20 using the following method (see notes 2 and 3 and fig. 1):
- Dispense:
  - 190 µl of "dilution buffer 2" per well
  - 10 µl of undiluted negative control in A1
  - 10 µl of undiluted positive control in B1 and C1
  - 10 µl of the serum to test in only one well
- 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 incubate for 1 hour (± 5 min.) at 37°C (± 3°C)

This method enables the test to be implemented in less than half a day.
Notes:
1. In our laboratories, a microplate shaker originally intended for a complement fixation test micromethod, is used.
2. The individual filling of the 96 wells is sometimes a long process. In order to standardize 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. It is nonetheless essential to make the dilutions of the samples in the same way as for the controls.
3. The position of the controls in A1, B1 and C1 is not important. They may be dispensed anywhere on the plate.
4. Laboratories, which use automatic methods, may not have enough reagents (i.e. "Concentrated X20 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 the crystals which previously 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" 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 with the "Dilution buffer 1".
b) Dispense 100 µl of diluted conjugate per well.
c) Cover the plate (with a lid, aluminium foil or adhesive) and incubate for 30 minutes (±3 min) at 37°C (±3°C).

4) WASHING
a) Empty the content of the plate by a “flick-off” or by another manual or automatic method.
b) Fill all the wells of the plate with the Wash solution; then empty them again.
c) Repeat step b) twice (a 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 a dry towel in order to empty completely the wells.

5) REVELATION
a) Dispense 100 µl of "Revelation Solution 3" ready to use per well
b) Leave to incubate the plates 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-minute revelation period, which is outlined above, gives the O.D. values provided in the paragraph "INTERPRETATION", when implemented in our laboratories. However, 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...). Regarding the work conditions, the revelation step may give OD values higher or lower than those expected. So, the user may stop the reaction at 20 minutes ± 10 min.

2. The reading can be done up to 1 hour after having stopped the reaction on condition that the plates are kept in the dark.

6) READING

a) 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 positive control has a minimal mean OD.450 value of: 0.350

and

A ratio between the mean OD.450 value of the positive control and the OD.450 value of the negative control is equal or greater than 3.5.

INTERPRETATION FOR SERA

Calculate for each sample, the S/P percentage:

\[
S/P \% = \frac{(\text{OD.450 value of the sample} - \text{OD.450 value of the negative control})}{(\text{mean OD.450 value of the positive control} - \text{OD.450 value of the negative control})} \times 100
\]

- Any sample with a \( S/P \% \leq 45\% \) is considered coming from an animal, which has not been in contact with the BHV-1 virus.
- Any sample with a \( S/P \% \) between 45% and 55% is considered to be doubtful (see note 1)
- Any sample with a \( S/P \% \geq 55\% \) is considered coming from an animal, which has been in contact with the BHV-1 virus.

Note:
1. It is advised to confirm the sera found doubtful with an ELISA Test including a control well or an ELISA using the competition principle (please contact us in order to know the corresponding batch numbers).
2. The use of active or inactivated vaccines induces a significant serological reaction.
3. Colostrum antibodies are detectable in calves up to the age of 6 month.
USING INSTRUCTIONS FOR MILK SAMPLES

1) DEPOSITING THE SAMPLES

a) Treatment of the samples:

• Control samples:
  Dilute the controls to 1/20 using the following method (see notes 2 and 3 and fig. 1):
  - Dispense:
    - 190 µl of "dilution buffer 2" per well
    - 10 µl of undiluted negative control in A1
    - 10 µl of undiluted positive control in B1 and C1

• Milk samples to test
  Dilute the milk samples to test to 1/2 using the following method (see notes 2 and 3 and fig. 1):
  - Dispense:
    - 100 µl of "dilution buffer 2" per well
    - 100 µl of the milk to test in only one well

b) Incubation of samples:
  - 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 incubate for 1 hour (± 5 min.)
    at 37°C (± 3°C)

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

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

Figure 1: Distribution of the Samples

N = Negative Control
P = Positive Control
1 = Sample no. 1
2 = Sample no. 2
3 = ........................................

Notes:
1. In our laboratories, a microplate shaker originally intended for a complement fixation test micromethod, is used.
2. The individual filling of the 96 wells is sometimes a long process. In order to standardize the sample 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. If is nonetheless essential to make the dilutions of the samples in the same way as for the controls.
3. The position of the controls in A1, B1 and C1 is not important. They may be dispensed anywhere on the plate.
4. Laboratories, which use automatic methods, may not have enough reagents (i.e. "Concentrated X20 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 the crystals which previously 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” 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 with the “Dilution buffer 1”.
   b) Dispense 100 µl of diluted conjugate per well.
   c) Cover the plate (with a lid, aluminium foil or adhesive) and incubate for 30 minutes (±3 min) at 37°C (±3°C).

4) WASHING
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   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 a dry towel in order to empty completely the wells.

5) REVELATION
   a) Dispense 100 µl of “Revelation Solution 3” ready to use per well
   b) Leave to incubate the plates 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-minute revelation period, which is outlined above, gives the O.D. values provided in the paragraph “INTERPRETATION”, when implemented in our laboratories. However 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...). Regarding the work conditions, the revelation step may give OD values higher or lower than those expected. So, the user may stop the reaction at 20 minutes ± 10 min.
   2. The reading can be done up to 1 hour after having stopped the reaction on condition that the plates are kept in the dark.

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   a) 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:

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and

A ratio between the mean OD.450 value of the positive control and the OD.450 value of the negative control is equal or greater than 3.5.

INTERPRETATION FOR MILK

Calculate for each sample, the S/P percentage:

\[
S/P\% = \frac{(\text{OD.450 value of the sample} - \text{OD.450 value of the negative control})}{(\text{mean OD.450 value of the positive control} - \text{OD.450 value of the negative control})} \times 100
\]

- Any sample with a S/P % ≤ 25% is considered coming from a group of animals, which has not been in contact with the BHV-1 virus.

- Any sample with a S/P % > 25% is considered coming from a group of animals, which has been in contact with the BHV-1 virus.

Note:
1. It is advised to confirm the sera found doubtful with an Elisa Test including a control well or an ELISA using the competition principle (please contact us in order to know the corresponding batch numbers).
2. The use of active or inactivated vaccines induces a significant serological reaction.
3. Colostrum antibodies are detectable in calves up to the age of 6 month.

LEGEND

 Modification in the using instructions

BIBLIOGRAPHY

