MAEDI-VISNA / C.A.E.V SERODIAGNOSIS

ELISA TEST SERUM

(240 REACTIONS)
**INTRODUCTION**

Maedi-Visna is a viral disease of sheep caused by lentivirus. It is characterized by progressive interstitial pneumonia or meningoencephalitis. Sometimes, arthritis and chronic mastitis are observed. The disease most often progresses slowly, but irreversibly. Its economic impact cannot be ignored.

Viral caprine arthritis encephalitis is a disease of adult goats caused by a RNA lentivirus (CAEV), leading to the appearance of arthritis (swelling in the knees) and mastitis. More rarely, CAEV can cause encephalitis in kids. CAEV is a disease which develops slowly, but irreversibly, and has an undeniable economic impact.

As no treatment or vaccine is available at the present time for these both diseases, the eradication programs are based on early detection accompanied by draconian sanitary measures (elimination of animals carrying the virus).

Serological screening is the easiest technique to implement on a large scale. Initially based on the agar-gel immunodiffusion technique, this serological diagnosis is now completed by ELISA technology, which is easier to use and to interpret and offering better detectability.

The ELISA technique proposed in this kit is an indirect ELISA based on the use of an immunogenic peptid of a trasmembrane protein (TM, ENV gene) and of the recombinant P28 protein which enters into the composition of the viral capsid (GaG gene). The appearance of anti-P28 antibodies can occur slightly later than that of the anti-viral envelop protein antibodies. The use of this very stable protein allows the serological detection of a very wide spectrum of serological variants.

**PRINCIPLE OF THE TEST**

The principle of the test is:

1. The wells of the microplates are coated with viral antigen (only the wells of the even-numbered columns are coated with the specific antigen).
2. Sera samples to be tested are diluted and incubated in the wells. Any antibodies specific to the antigen present in the serum will form an antigen-antibodies (ovine/caprine) immune-complex and remains bound in the wells.
3. After washing, an antibody directed to ovine/caprine IgG and linked to an enzyme (peroxydase) is added to incubate. This conjugate will bind to the immune-complex.
4. After washing, the enzyme substrate (TMB) is added to the wells. If the immune-complex is present the peroxydase transforms 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 serum sample to be tested.

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

**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. Microplate washing system that distributes 300 µl per well
6. Precision Micropipettes and Multi-dispensing micropipettes (The precision required must be lower or equivalent to 10% for volumes lower or equivalent 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 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...).
9. Microplate covers
10. Incubator at +37°C (±3°C)
**KIT CONTENTS and STORAGE OF REAGENTS**

It is recommended to work with all components at 21°C (± 5°C). Thus all reagents must be brought to room temperature at least one hour before the beginning of the test (except the conjugate and the control samples).

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>QUANTITY</th>
<th>STORAGE AND NOTES</th>
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<tbody>
<tr>
<td>Biowell coated microplates</td>
<td>5</td>
<td>+5°C (± 3°C)</td>
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<td></td>
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<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>
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<tr>
<td>Concentrated (X 20) wash solution</td>
<td>1 x 100 ml bottle</td>
<td>+5°C (± 3°C)</td>
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<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>
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<td>• This solution can also be stored at 21°C (± 5°C) up to 1 month, if the vials are closed in an airtight way, and be immediately available when needed.</td>
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<td>• After dilution, the Wash Solution can be stored for 3 days at +5°C (± 3°C).</td>
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<td>• The &quot;Concentrated (X 20) wash solution&quot; is similar for all the kits of INSTITUT POURQUIER and can be used equally in the different kits.</td>
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<tr>
<td>Dilution Buffer 4 Purple (for samples)</td>
<td>1 x 120 ml bottle</td>
<td>+5°C (± 3°C)</td>
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<td>Dilution Buffer 1 blue (for conjugate)</td>
<td>1 x 120 ml bottle</td>
<td>+5°C (± 3°C)</td>
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<td>Positive control (liquid)</td>
<td>1 x 0.5 ml bottle</td>
<td>+5°C (± 3°C)</td>
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<tr>
<td>Negative control (liquid)</td>
<td>1 x 0.5 ml bottle</td>
<td>+5°C (± 3°C)</td>
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<tr>
<td>Anti-ruminants IgG-peroxydase conjugate</td>
<td>1 x 0.75ml bottle</td>
<td>+5°C (±3°C)</td>
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<td>• The diluted conjugate solution cannot be stored.</td>
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<tr>
<td>Revelation Solution 2 (TMB) Ready to use</td>
<td>1 x 60 ml bottle</td>
<td>+5°C (± 3°C).</td>
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<tr>
<td>Stop Solution (H₂SO₄ 0.5M solution)</td>
<td>1 x 120 ml bottle</td>
<td>+5°C (± 3°C)</td>
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<td>• It can be stored at +21°C (± 5°C) up to 1 month if the vial is well closed in an airtight manner and be immediately available when needed.</td>
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<td>• The &quot;Stop Solution&quot; is similar for all the kits of INSTITUT POURQUIER and can be used equally in the different kits.</td>
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</table>

**PRECAUTIONS FOR USE**

1. Do not place the pipette in the mouth when testing reagents.
2. "Stop-solution" contains H₂SO₄*(0,5M) acid, that can cause serious burns in case of contacts with skin, mucous membranes and eyes.
3. Even if the material delivered in the package does not contain any contaminating element, and that the serum 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.

* The toxicity form of the product is available at the Institut Pourquier

Institut POURQUIER

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INSTRUCTIONS FOR USE

1) DEPOSITING THE SERA

Sera (controls and samples) are diluted to 1/20 and incubated for 1 hour (± 5 min.) at 37°C (±3°C) using the following method:

⇒ Dispense:
  • 190 µl of “dilution buffer 4" per well.
  • 10 µl of undiluted negative control serum in A1 and A2
  • 10 µl of undiluted positive control serum in B1 / B2 and C1 / C2

⇒ Dispense 10 µl of each undiluted serum sample in (see fig.1):
  • 1 coated well (even-numbered column)
  • 1 uncoated well (odd-numbered column)

⇒ Homogenize the contents of the wells by delicately 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).

\[\text{Figure 1: Distribution of the Sera}\]

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Notes:
1) In our laboratories, a microplate shaker originally designed 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 wells with U-shaped-bottoms. It is therefore possible to transfer them rapidly (column by column) by using a multi-channel pipette.
3) The position of controls in A1, A2 , B1, B2, C1 and C2 is not important, they may be dispensed anywhere on the plate. It may be better to add replicates of controls to the same plate in order to establish an average OD value. It is advised to place one positive and one negative control in the middle of the plate. Therefore it is necessary to work in the same way with the sera to be tested as with the controls.
4) Sometimes, laboratories which use automatic methods 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 the crystals which previously appeared at +5°C (±3°C), as long as the whole 100 ml vial is used.

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

c) Fill all the wells on the plate with the “wash solution”, then empty them again.

d) Repeat 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 this solution per well.

c) Cover the plate (with a lid or aluminium foil) and incubate for 30 minutes (±3 min.) at 37°C (±3°C).

### 4) WASHING

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

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

c) Repeat the step b) twice (total of 3 washes)

**Note:**
The care brought to the last washing is essential for a good implementation of the test. If the washing is manual, the plate can be inverted and tapped dry on absorbent support, in order to completely empty the wells after the last washing.

### 5) REVELATION

a) Dispense 100 µl of "Revelation Solution 2" ready to use 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 - 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 altered by different factors (quality of the washes, quality of water used, precision of the pipetting, temperature of the reaction...). So, the user may stop the reaction several minutes before or after the indicated time.

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.

b) Calculate the corrected OD 450 for each serum: 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 positive control serum has a minimal mean uncorrected OD 450 value of **0.350**

A ratio between the mean 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 «negative» or equal to zero. In that case, use the absolute value for the validation.
**INTERPRETATION**

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

$$S/P = \frac{\text{corr. OD450 value of the sample}}{\text{mean corrected OD450 value of the positive control}} \times 100$$

**MAEDI-VISNA and CAEV APPLICATIONS**

- Samples with a $S/P\%$ equal or lower than **110\%** are considered coming from animals which have not been in contact with the MAEDI-VISNA or CAEV viral antigens.

- Samples with a $S/P\%$ between **110\%** and **120\%** are considered doubtful (see note).

- Samples with a $S/P\%$ equal or higher than **120\%** are considered coming from animals which have been in contact with the MAEDI-VISNA or CAEV viral antigens.

**Note**: samples showing a $S/P\%$ between **110\%** and **120\%** must be confirmed by a second test. If after this second test, the samples present again a $S/P\%$ between the same values, these sera will be considered as doubtful. In that case, it is advised to confirm these results with a new blood sample.

**LEGEND**

 harmed face : Modification in the using instructions

! : Minor modification in the text