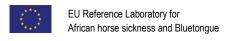


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LABORATORIO CENTRAL DE VETERINARIA





OIE Reference Laboratory for African horse sickness

| DETECTION OF ANTIBODIES AGAINST BLUETONGUE USING A COMPETITIVE/BLOCKING ELISA METHOD |                          |         |
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#### 1. SCOPE

To detect the presence of antibodies against Bluetongue virus in serum samples of domestic and wild ruminants using competitive/blocking ELISA commercial kits.

#### 2. MATERIALS AND EQUIPMENT

Material required but not provided in the kit

Distilled or deionized water (ELIX®)

Disposable pipette tips

Graduated cylinder for wash solution

**Graduated pipettes** 

Bucket to dispense reagents

Microplate adhesive covers

Waste bags

Internal control (weak positive serum) - recommended

#### Equipment

Freezer -20°C (<-18°C)

Cooler +5°C (+2<T°2<+8°C)

Precision micropipettes or multi-dispensing micropipettes

**Automatic Pipettor** 

Vortex

Microplate shaker

Microcentrifuge

Incubators (37 ± 2° C) and (18° - 25°C)

Chronometer

Microplate washer (manual)

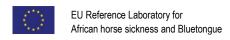
Spectrophotometre (96-well microplate reader with 450 nm filter)

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Biosafety cabinet type II (if handling of samples requires it)

#### Kit composition

Reagents included in each ELISA kit are described in the Annexes

#### 3. METHOD

The principle of the competitive/blocking (c/b) ELISA test is to avoid the reaction between the recombinant VP7 protein (serogroup specific) adsorbed to the ELISA plate and a conjugated with peroxidase serogroup-VP7 specific monoclonal antibody (Mab), by means of the specific anti-VP7 antibodies that could be present into the sample. Antibodies in tested serum will block the reaction between the antigen and the Mab resulting in a reduction in colour in comparison with that obtained in negative control serum.

VP7, whose sequence is relatively well-conserved between isolates, is the most abundant structural protein and the major immunogenic serogroup-reactive protein. Because the conjugate is a Mab and it is directed against the VP7, the assay will give a high level of sensitivity and specificity.

There are several commercial kits based on this principle using one of a number of BT serogroup-reactive MAbs. Genetic divergence of certain BTV strains (e.g. different regional groups or topotypes) may affect the nature of serogroup-reactive antibodies. It is therefore possible that diagnostic characteristics for antibody detection are not uniform for all viruses encompassed by the serogroup.

For this reason there have to be a technical decision to choose the most adequate ELISA kit depending on the epidemiological situation.

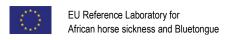
The protocol for each c/b ELISA is described in the corresponding Annex.

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#### 4. ANNEXS

ANNEX 1 cELISA Procedure: ID Screen BTV Competition

ANNEX 2 cELISA Procedure: IDEXX Bluetongue Competition

ANNEX 3 bELISA Procedure: Ingezim AHSV Compac Plus

#### 5. REFERENCES

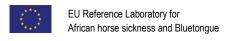
OIE. Manual for Diagnostic Tests and Vaccines for Terrestrial Animals. English version in force at date.

Chapter: Bluetongue (Infection with Bluetongue virus)



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#### ANNEX 1. cELISA Procedure: ID Screen Bluetongue Competition

Based on ID Screen BTV Competition: Competition enzyme-linked immunosorbent assay for detection of specific antibodies to bovine, ovine and caprine, as well as other wild ruminants, in serum samples. ID VET. Manufacturer's instruction manual.

#### KIT COMPOSITION

- a. BTV-VP7 coated plates (96 well microplates divided in strips 12x8)
- b. Positive control
- c. Negative control
- d. Conjugate anti-VP7-HRP concentrated 10X
- e. Dilution buffer nº2
- f. Wash solution concentrated 20X
- g. TMB (3,3',5,5'-Tetramethylbenzidine) Substrate (ready to use)
- h. Stop solution 0,5M (ready to use)

Components "b", "c", "d" and "g" must be stored at +5°C (±3°C). The other components can be stored at room temperature.

Components "e" and "f" from any other ID VET kit could be used.

#### PREPARATION OF REAGENTS

Wash solution (1X): wash solution 20X must be diluted 1:20 with distilled/deionized water (e.g. 15 ml of wash solution concentrated 20X in 285 ml distilled water). It is stable when stored at 5°C (±3°C) for a week, labelled including the expiry date.

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Conjugate anti-VP7-HRP (1X): conjugate anti-VP7-HRP concentrated 10X must be diluted 1:10 with Dilution buffer nº 2 just before to use (e.g. 1 ml of conjugate concentrated 10X in 9 ml of Dilution buffer nº 2).

#### **TEST PROCEDURE**

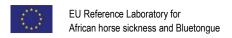
- ✓ All reagents and samples must be allowed to come to room temperature before use. Mix reagents by gentle inverting or swirling.
- ✓ Obtain coated plates and record the sample position in the template. If using partially the plate, record a number in each strip to order in case of strip fall down during the washing.
- ✓ Dispense 50 µl of Dilution buffer nº2 in each well.
- ✓ Dispense 50 µl of Positive control in A1 and B1.
- ✓ Dispense 50 µl of Negative control in C1 and D1.
- ✓ Dispense 50 µl of each sample per well.
- ✓ According to Guideline for ELISA, it is strongly recommended to include a weak positive serum as control
- ✓ Mix the content of the microwells by gently tapping the plate or use a microplate shaker. Cover the plate and incubate 45 minutes ± 4 min. at 21°C (±5°C).
- ✓ Dispense 100 μl of Conjugate anti-VP7-HRP 1X. IMPORTANT: Don't remove the solution in the plate neither washing before adding the conjugate.
- ✓ Incubate 30 minutes ± 3 min. at 21°C (±5°C).
- ✓ Remove the solution and wash each well with approximately 300 μl of Wash solution 1X, 3 times. Avoid plate drying prior to the addition of the next reagent.
- ✓ Dispense 100 µl of Substrate TMB (ready-to-use) in each well.
- ✓ Incubate 15 minutes ± 2 min. at 21°C (±5°C) away from light.
- ✓ Dispense 100 µl of Stop solution 0,5M (ready-to-use) in each well.
- ✓ Measure in a Spectrophotometer (96-well microplate reader) at 450 nm.

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#### **RESULT INTERPRETATION**

#### Validity criteria:

- -Average OD <sub>Negative control</sub> > 0,7
- -(Average OD Positive control / Average OD Negative control) < 0,3
- -Weak positive internal control should have the expected result.

NOTE: According to Guideline for ELISA, it is recommended to monitor the repeatability between replicates in positive and negative controls.

#### **Interpretation**:

To calculate (S/N%) in each sample:

S/N%= (OD Sample / OD Negative control) x 100

If S/N% <  $40\% - \rightarrow$  **POSITIVE** 

If  $S/N\% \ge 40\% - \rightarrow NEGATIVE$ 

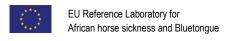
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#### ANNEX 2. cELISA Procedure: IDEXX Bluetongue Competition

Based on *IDEXX Bluetongue Competition*: Competition enzyme-linked immunosorbent assay for detection of specific antibodies to bovine, ovine and caprine, as well as other wild ruminants, in serum samples. IDEXX. Manufacturer's instruction manual.

#### KIT COMPOSITION

BTV-VP7 coated plates (96 well microplates divided in strips 12x8)

Positive control

Negative control

Conjugate anti-VP7-HRP concentrate 20X

Dilution buffer nº2

Wash solution concentrated 20X

TMB (3,3',5,5'-Tetramethylbenzidine) Substrate nº 12 (ready to use)

Stop solution nº 3 (ready to use)

Components must be stored at +5°C (±3°C).

#### **PREPARATION OF REAGENTS**

Wash solution (1X): Wash solution concentrated 20X must be diluted 1:20 with distilled/deionized water (e.g. 15 ml of wash concentrated 20X in 285 ml distilled water). It is stable for up 3 days when stored at 5°C (±3°C), labelled including the expiry date.

Conjugate anti-VP7-HRP (1X): must be diluted 1:20 in the Wash solution 1X. It is stable for up 8 hours when stored at 21°C (±5°C). (e.g. 1 ml of conjugate concentrated 20X in 19 ml of Wash solution 1X)

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# **DETECTION OF ANTIBODIES AGAINST BLUETONGUE USING A** COMPETITIVE/BLOCKING ELISA METHOD

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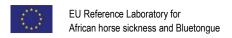
#### **TEST PROCEDURE**

- ✓ All reagents and samples must be allowed to come to room temperature before use. Mix reagents by gentle inverting or swirling.
- ✓ Obtain coated plates and record the sample position in the template. If using partially the plate, record a number in each strip to order in case of strip fall down during the washing.
- ✓ Dispense 80 µl of Dilution buffer nº2 in each well.
- ✓ Dispense 20 µl of ready-to-use Positive control in A1.
- ✓ Dispense 20 µl of ready-to-use Negative control in C1 and D1.
- ✓ Dispense 20 µl of each sample per well.
- ✓ According to Guideline for ELISA, it is strongly recommended to include a weak positive serum as control
- ✓ Mix the content of the microwells by gently tapping the plate or use a microplate shaker. Cover the plate and incubate 45 minutes ± 3 min. at 21°C (±5°C).
- ✓ Dispense 100 µl of Conjugate anti-VP7-HRP 1X.
- ✓ IMPORTANT: Don't remove the solution in the plate neither washing before adding the conjugate.
- ✓ Incubate 45 minutes ± 3 min. at 21ºC (±5ºC).
- Remove the solution and wash each well with approximately 300 μl of Wash solution 1X, 3 times. Avoid plate drying prior to the addition of the next reagent.
- ✓ Dispense 100 µl of Substrate TMB nº 12 (ready-to-use) in each well.
- ✓ Incubate 10 minutes ± 3 min. at 21°C (±5°C) away from light.
- ✓ Dispense 100 µl of Stop solution nº 3 (ready-to-use) in each well.
- ✓ Measure in a Spectrophotometer (96-well microplate reader) at 450 nm.



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#### **RESULT INTERPRETATION**

<u>Calculate S/N%</u> of samples and Positive control:

S/N%= 100 x (OD Sample or Positive control / Average OD Negative control )

#### Validity criteria:

- 3,0 ≥ Average OD <sub>Negative control</sub> ≥ 0,7
- S/N% Positive control < 20%
- -Weak positive internal control should have the expected result.

NOTE: According to Guideline for ELISA, it is recommended to monitor the repeatability between replicates in negative and positive controls.

#### **Interpretation**:

If  $S/N\% \ge 80 - \rightarrow NEGATIVE$ 

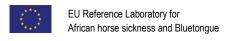
If S/N%  $\leq$  70 -  $\rightarrow$  **POSITIVE** 

If  $70 < S/N\% < 80 \rightarrow DOUBTFUL$ 



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#### ANNEX 3. bELISA Procedure: Ingezim BTV Compac 2.0

Based on *Ingezim BTV Compac 2.0*: Blocking enzyme-linked immunosorbent assay for detection of specific antibodies to bovine, ovine and caprine, as well as other wild ruminants, in serum samples. INGENASA. Manufacturer's instruction manual.

#### KIT COMPOSITION

BTV-VP7 coated plates (96 well microplates divided in strips 12x8)

Positive control

**Negative control** 

Conjugate (Mab anti-VP7-HRPO) (ready-to-use)

Dilution buffer

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Wash solution concentrated 25X

TMB (3,3',5,5'-Tetramethylbenzidine) Substrate (ready to use)

Stop solution (ready to use)

Components must be stored at +5°C (±3°C).

#### **PREPARATION OF REAGENTS**

Wash solution (1X): Wash solution concentrated 25X must be diluted 1:25 with distilled/deionized water (e.g. 40 ml of wash concentrated 25X in 960 ml distilled water). It is stable when stored at 5°C (±3°C) for a week, labelled including the expiry date.



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#### **TEST PROCEDURE**

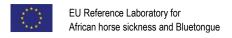
- ✓ All reagents and samples must be allowed to come to room temperature before use. Mix reagents by gentle inverting or swirling.
- ✓ Obtain coated plates and record the sample position in the template. If using partial plate, record a number in each strip to order in case of strip fall down during the washing.
- ✓ Dispense 50 µl of Dilution buffer in each well (included positive and negative control wells)
- ✓ Dispense 50 µl of Positive control in A1 and B1.
- ✓ Dispense 50 µl of Negative control in C1 and D1.
- ✓ Dispense 50 µl of each sample per well.
- ✓ According to Guideline for ELISA, it is strongly recommended to include a weak positive serum as control
- ✓ Mix the content of the microwells by gently tapping the plate or use a microplate shaker. Cover the plate and incubate overnight (16-18 hours) at room temperature (18 - 25°C) or alternatively 180 minutes at 37ºC.
- ✓ Remove the solution and wash each well with approximately 300 µl of Wash solution 1X, 6 times. Avoid plate drying prior to the addition of the next reagent.
- ✓ Dispense 100 µl of Conjugate ready-to-use.
- ✓ Seal the plate and incubate 30 minutes at 37°C.
- ✓ Remove the solution and wash each well with approximately 300 μl of Wash solution 1X, 6 times. Avoid plate drying prior to the addition of the next reagent.
- ✓ Dispense 100 µl of Substrate TMB (ready-to-use) in each well.
- ✓ Incubate 10 minutes at 21°C (±5°C) away from light.
- ✓ Dispense 100 µl of Stop solution (ready-to-use) in each well.
- ✓ Measure in a Spectrophotometer (96-well microplate reader) at 450 nm.

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#### **RESULT INTERPRETATION**

#### Validity criteria:

- (Average OD Positive control / Average OD Negative control ) <0,25
- Weak positive internal control should have the expected result.

NOTE: According to Guideline for ELISA, it is recommended to monitor the repeatability between replicates in positive and negative controls.

#### **Interpretation**:

To calculate:

Cut off (-): 0,65 x Average OD Negative control

Cut off (+): 0,60 x Average OD Negative control

To calculate % Blocking in each sample:

Blocking %= 100 - [(OD Sample x 100) / Average OD Negative control]

If OD <sub>Sample</sub>  $\leq$  Cut off (+)  $\rightarrow$  **POSITIVE** (Blocking %  $\geq$  40)

If OD <sub>Sample</sub>  $\geq$  Cut off (-)  $\rightarrow$  **NEGATIVE** (Blocking %  $\leq$  35)

If Cut off (-) < OD <sub>Sample</sub> < Cut off (+)  $\rightarrow$  **DOUBTFUL** (40 > Blocking % > 35)