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

Date: 22/06/2021	Document code: GL-LCV-05	*Rev. 02*

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 -20ºC (<-18ºC)

Cooler +5ºC (+2<Tª<+8º C)

Precision micropipettes or multi-dispensing micropipettes

Automatic Pipettor

Vortex

Microplate shaker

Microcentrifuge

Incubators (37 ± 2º C) and (18º – 25ºC)

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 +5ºC (±3ºC). 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 5ºC (±3ºC) 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 21ºC (±5ºC).
- ✓ 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 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 (ready-to-use) in each well.
- ✓ Incubate 15 minutes ± 2 min. at 21ºC (±5ºC) 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 $_{\text{Negative control}}$ > 0,7

-(Average OD $_{\text{Positive control}}$ / Average OD $_{\text{Negative control}}$) < 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 $_{\text{Sample}}$ / OD $_{\text{Negative control}}$) x 100

If S/N% < 40% -→ **POSITIVE**

If S/N% ≥ 40% -→ **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 +5ºC (±3ºC).

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 5ºC (±3ºC), 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 21ºC (±5ºC). (e.g. 1 ml of conjugate concentrated 20X in 19 ml of Wash solution 1X)

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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 21ºC (±5ºC).
- ✓ 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 21ºC (±5ºC) 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

Calculate S/N% of samples and Positive control:

S/N%= 100 x (OD $_{\text{Sample or Positive control}}$ / Average OD $_{\text{Negative control}}$)

Validity criteria:

- 3,0 ≥ Average OD $_{\text{Negative control}}$ ≥ 0,7
- S/N% $_{\text{Positive control}}$ < 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% ≥ 80 -→ **NEGATIVE**

If S/N% ≤ 70 -→ **POSITIVE**

If 70 < S/N% < 80 → **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

Wash solution concentrated 25X

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

Stop solution (ready to use)

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

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 5ºC (±3ºC) 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 – 25ºC) 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 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 Substrate TMB (ready-to-use) in each well.
- ✓ Incubate 10 minutes at 21ºC (±5ºC) 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 $_{\text{Positive control}}$ / Average OD $_{\text{Negative control}}$) <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 $_{\text{Negative control}}$

Cut off (+): 0,60 x Average OD $_{\text{Negative control}}$

To calculate % Blocking in each sample:

Blocking %= 100 - [(OD $_{\text{Sample}}$ x 100) / Average OD $_{\text{Negative control}}$]

If OD $_{\text{Sample}}$ ≤ Cut off (+) → **POSITIVE** (Blocking % ≥ 40)

If OD $_{\text{Sample}}$ ≥ Cut off (-) → **NEGATIVE** (Blocking % ≤ 35)

If Cut off (-) < OD $_{\text{Sample}}$ < Cut off (+) → **DOUBTFUL** (40 > Blocking % > 35)

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