DETECTION OF ANTIBODIES AGAINST BLUETONGUE USING A DOUBLE RECOGNITION ELISA METHOD

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

1. SCOPE

To detect the presence of antibodies against Bluetongue virus in sera samples of domestic and wild ruminants using a Double Recognition ELISA commercial kit.

Although double recognition (dr) ELISA has shown to be reliable and useful for BT antibodies detection; it is not included in the OIE Manual at the moment.

2. MATERIALS AND EQUIPMENT

Material required but not provided in the kit

Distilled or deionized water

Disposable pipette tips

Graduated cylinder for wash solution

Graduated pipettes

Bucket to dispense reagents

Microplate adhesive covers

Waste bags

Weak positive 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

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Incubators (37 ± 2º C and 18 – 25º)

Chronometer

Microplate washer (manual)

Spectrophotometre 96-well microplate reader (450 nm filter)

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

Kit composition

The reagents included in each ELISA kit are described in the Annex 1

Store these reagents at 2 – 8ºC

3. METHOD

The drELISA used in Bluetongue serological diagnostic is based on recombinant VP7 protein (serogroup specific) adsorbed to the ELISA plate (the same that c/bELISA). The antibodies in the serum sample binded to the VP7 protein are detected using the same recombinant VP7 protein conjugated with peroxidase. Presence or absence of labelled VP7 will be detected by addition of substrate (TMB) which, in presence of peroxidase, will develop a colorimetric reaction. There are commercial kits based on this principle.

4. ANNEXES

ANNEX 1 drELISA Procedure: *Ingezim BTV DR*

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ANNEX 1 drELISA Procedure: *Ingezim BTV DR*

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

KIT COMPOSITION

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

Positive control (ready-to-use)

Negative control (ready-to-use)

Conjugate VP7-HRP (ready-to-use)

Dilution buffer (DE01-01)

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 solution 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 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 in wells where samples to be assayed.
- ✓ Dispense 100 µl of Positive control in A1 and B1.
- ✓ Dispense 100 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 internal control
- ✓ Mix the content of the microwells by gently tapping the plate or use a microplate shaker. Cover the plate and incubate 60 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 Conjugated VP7-HRP ready-to-use.
- ✓ Seal the plate and incubate 60 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 15 minutes at 21ºC (±5ºC) away from light.
- ✓ Dispense 100 µl of Stop solution (ready-to-use) in each well.
- ✓ Measure in a Spectrophotometre (96-well microplate reader) at 450 nm. within 5 min after the addition of stop solution.

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RESULT INTERPRETATION

Cut off calculation:

0,15 x $OD_{Positive\ control}$

Validity criteria:

- Average $OD_{Positive\ control} > 0,8$
- Average $OD_{Negative\ control} <$ Cut off
- 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:

If OD value > cut off -→ **POSITIVE** (there are specific antibodies against BTV)

If OD value ≤ cut off -→ **NEGATIVE** (there are not specific antibodies against BTV)

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