DETECTION OF ANTIBODIES AGAINST AFRICAN HORSE SICKNESS USING A BLOCKING ELISA METHOD

Date: 22/06/2021	Document code: GL-LCV-04	*Rev. 02*

1. SCOPE

To detect the presence of antibodies against African horse sickness (AHS) virus in equine serum samples using a blocking ELISA commercial kit.

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 (serum)

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

Chronometer

Microplate washer (manual)

Spectrophotometre (96-well microplate reader with 405 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 Annex

3. METHOD

VP7 is the main antigenic protein within the molecular structure of AHSV and it is highly conserved across the nine AHSV serotypes. A monoclonal antibody (MAb) directed against VP7 is used in this test, allowing high sensitivity and specificity. Moreover, other species of equidae (e.g. donkeys, zebra, etc.) can be tested thus preventing the problem of specificity experienced occasionally using the indirect ELISAs. VP7 recombinant antigen is non-infectious, which provides a high level of biosafety.

The principle of this test is to block the specific reaction between the recombinant VP7 protein adsorbed on an ELISA plate and a conjugated MAb against VP7. AHSV antibodies present in a suspect serum sample will block this reaction. A decrease in the amount of colour in comparison with negative control is evidence of the presence of AHSV antibodies in the serum sample.

There is only one available commercial kit based on this principle using AHSV serogroup-reactive MAbs. The protocol for this bELISA is described in the corresponding Annex.

4. ANNEXES

ANNEX 1 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 African horse sickness (Infection with African horse sickness virus)

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ANNEX 1. bELISA Procedure: *Ingezim AHSV Compac Plus*

Based on *Ingezim AHS Compac Plus*: Blocking enzyme-linked immunosorbent assay for detection of specific antibodies of specific antibodies to AHS Virus in equine sera. INGENASA. Manufacturer's instruction manual.

KIT COMPOSITION

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

Positive control (ready to use)

Negative control (ready to use)

Conjugate anti-VP7-HRP concentrated (ready-to-use)

Dilution buffer

Wash solution concentrate 25X

ABTS S (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) 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 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 in each well (except in the negative and positive control wells)
- ✓ Dispense 100 µl of ready to use Positive control in A1 and B1.
- ✓ Dispense 100 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 internal control
- ✓ Mix the content of the microwells by gently tapping the plate or use a microplate shaker. Cover the plate and incubate for 1 hour at 37ºC.
- ✓ Remove the solution and wash each well with approximately 300 µl of Wash solution 1X, 5 times. Avoid plate drying prior to the addition of the next reagent.
- ✓ Dispense 100 µl of Conjugate anti-VP7-HRP 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, 5 times. Avoid plate drying prior to the addition of the next reagent.
- ✓ Dispense 100 µl of Substrate ABTS (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 96-well microplate at 405 nm.

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RESULT INTERPRETATION

Validity criteria:

- Average OD $_{\text{Positive control}}$<0,2
- Average OD $_{\text{Negative control}}$) >1
- 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 % Blocking in each sample:

Blocking %= [(OD $_{\text{Neg. Control}}$ - OD $_{\text{Sample}}$) / (OD $_{\text{Neg. Control}}$ - OD $_{\text{Pos. Control}}$)] x 100

If Blocking % > 50 → **POSITIVE**

If Blocking % < 45 → **NEGATIVE**

If 45 ≤ Blocking % ≤ 50 → **DOUBTFUL**

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