Council Directive 2009/156/EC of 30 November 2009 on animal health conditions governing the movement and importation from third countries of equidae (codified version) (Text with EEA relevance)

[F1ANNEX IV

AFRICAN HORSE SICKNESS DIAGNOSIS

Textual Amendments

F1 Substituted by Commission Implementing Decision (EU) 2016/1840 of 14 October 2016 amending Annex IV to Council Directive 2009/156/EC as regards methods for African horse sickness diagnosis (notified under document C(2016) 6509) (Text with EEA relevance).

PART A

Serological tests

2. Blocking ELISA for the detection of antibodies to African horse sickness virus (AHSV)

The competitive blocking ELISA is designed to detect specific AHSV antibodies in sera from animals of any equine species, i.e. horses, donkeys, zebra and their crosses, preventing the problem of specificity experienced occasionally using the indirect ELISAs.

The principle of the test is the blocking of the reaction between the recombinant VP7 protein absorbed to the ELISA plate and a conjugated AHS-VP7 specific monoclonal antibody (Mab). Antibody in the test sera will block the reaction between the antigen and the Mab resulting in a reduction in colour. Because the Mab is directed against the VP7, the assay will give a high level of sensitivity and specificity.

The competitive blocking ELISA is commercially available.

2.1. *Test procedure*

2.1.1. Solid Phase

2.1.1.1. Coat ELISA plates with 50-100 ng of recombinant AHSV-4 VP7 diluted in carbonate/bicarbonate buffer, pH 9,6. Incubate overnight at 4 °C.

2.1.1.2. Wash the plates three times with phosphate buffered saline (PBS) 0,1× containing 0,135 M NaCl and 0,05 % (v/v) Tween 20 (PBST). Gently tap the plates on to absorbent material to remove any residual wash.

2.1.2. Test samples and controls

2.1.2.1. Serum samples to be tested, and positive and negative control sera, are diluted 1 in 5 in diluent containing 0,35 M NaCl, 0,05 % (v/v) Tween 20 and 0,1 % Kathon, 100 µl per well. Incubate for 1 hour at 37 °C.

For titration, make a twofold dilution series of the test sera from 1 in 10 to 1 in 280 across 8 wells (100 µl/well), one serum per plate column, and do the same with positive and negative controls. Incubate for 1 hour at 37 °C.

2.1.2.2. Wash the plates five times with phosphate buffered saline (PBS) 0,1× containing 0,135 M NaCl and 0,05 % (v/v) Tween 20 (PBST). Gently tap the plates on to absorbent material to remove any residual wash.

2.1.3. Conjugate

2.1.3.1. Dispense 100 µl/well of horseradish peroxidase-conjugated Mab anti-VP7. In advance, this Mab has been diluted 1/5 000-1/15 000 in a 1/1 solution of StabiliZyme Select® Stabilizer (SurModics. Reference: SZ03) in distilled water. Incubate for 30 minutes at 37 °C.

2.1.3.2. Wash the plates five times with phosphate buffered saline (PBS) 0,1× containing 0,135 M NaCl and 0,05 % (v/v) Tween 20 (PBST). Gently tap the plates on to absorbent material to remove any residual wash.

2.1.4. Chromogen/Substrate

Add 100 µl/well chromogen/substrate solution, i.e. 1 ml of ABTS (2,2′-Azino-bis-[3-ethylbenzothiazoline-6-sulphonic acid]) 5 mg/ml + 9 ml of substrate buffer (0,1 M Phosphate-Citrate buffer of pH 4 containing 0,03 % H_2O_2), and incubate for 10 minutes at room temperature. Colour development is stopped by adding 100 µl/well of 2 % (w/v) SDS (sodium dodecyl sulphate).

2.1.5. Reading

Read at 405 nm in an ELISA reader.

2.2. *Interpretation of the results*

2.2.1. Determine the blocking percentage (BP) of each sample by applying the following formula, where ‘Abs’ stands for antibodies:

$$\mathrm{BP} = \frac{\mathrm{Abs(control^-)} - \mathrm{Abs(sample)}}{\mathrm{Abs(control^-)} - \mathrm{Abs(control^+)}} \times 100$$

2.2.2. Samples showing a BP value higher than 50 % should be considered as positive for AHSV antibodies.

2.2.3. Samples showing a BP value lower than 45 % should be considered as negative for AHSV antibodies.

2.2.4. Samples showing a BP value between 45 % and 50 % should be considered as inconclusive and must be retested. If the result is again inconclusive, the animals should be retested on samples taken not earlier than two weeks after the sample which was considered to be inconclusive was taken.**]**