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)

ANNEX I

COMPULSORILY NOTIFIABLE DISEASES

The following diseases are compulsorily notifiable:

— Dourine
— Glanders
— Equine encephalomyelitis (of all types, including VEE)
— Infectious anaemia
— Rabies
— Anthrax
— African horse sickness
— Vesicular stomatitis

ANNEX II

MODEL

HEALTH ATTESTATION[1]

Passport No …

I, the undersigned, certify that[2] the animal identified above meets the following requirements:

(a) it has been examined today and shows no clinical sign of disease;

(b) it is not intended for slaughter under a national programme of contagious or infectious disease eradication;

(c) — it does not come from the territory or part of the territory of a Member State which is the subject of restrictions for reasons of African horse sickness, or

it comes from the territory or part of the territory of a Member State which was subject to prohibition for animal health reasons and has undergone, with satisfactory results, the tests provided for in Article 5(5) of Directive 2009/156/EC in the quarantine station of … between …and…[3]

— it is not vaccinated against African horse sickness, or,

it was vaccinated against African horse sickness on …[3][4];

(d) it has not come from a holding which was subject to prohibition for animal health reasons nor had contact with equidae from a holding which was subject to prohibition for animal health reasons:

— during six months in the case of equidae suspected of having contracted dourine, beginning on the date of the last actual or possible contact with a sick animal. However, in the case of a stallion, the prohibition shall apply until the animal is castrated,

— during six months in the case of glanders or equine encephalomyelitis, beginning on the day on which the equidae suffering from the disease in question are slaughtered,

— in the case of infectious anaemia, until the date on which, the infected animals having been slaughtered, the remaining animals have shown a negative reaction to two Coggins tests carried out three months apart,

— during six months from the last case, in the case of vesicular stomatitis,

— during one month from the last case, in the case of rabies,

— during 15 days from the last case, in the case of anthrax,

— if all the animals of species susceptible to the diseases located on the holding have been slaughtered and the premises disinfected during 30 days, beginning on the day on which the animals were destroyed and the premises disinfected, except in the case of anthrax, where the period of prohibition is 15 days;

(e) to the best of my knowledge, it has not been in contact with equidae suffering from an infectious or contagious disease in the 15 days prior to this declaration;

(f) at the time of the inspection it was fit to be transported on the intended journey in accordance with the provisions of Regulation (EC) No 1/2005[(5)].

Date	**Place**	**Stamp and signature of the official veterinarian**[a]

a Name in block capitals and capacity.

ANNEX III

MODEL
HEALTH CERTIFICATE
For trade between Member States
EQUIDAE

EUROPEAN COMMUNITY **Intra trade certificate**

Part I: Details of consignment presented

I.1. Consignor Name Address Postal code		I.2. Certificate reference number	I.2.a. Local reference number:
		I.3. Central Competent Authority	
		I.4. Local Competent Authority	
I.5. Consignee Name Address Postal code		I.6. No(s) of related original certificates No.(s) of accompanying documents	
		I.7.	
I.8. Country of origin ISO code	I.9. Region of origin Code	I.10. Country of destination ISO code	I.11. Region of destination Code
I.12. Place of origin/Place of harvest Holding ☐ Assembly centre ☐ Other ☐ Name Approval number Address Postal code		I.13. Place of destination Holding ☐ Assembly centre ☐ Establishment ☐ Other ☐ Name Approval number Address Postal code	
I.14. Place of loading Postal code		I.15. Date and time of departure	
I.16. Means of transport Aeroplane ☐ Ship ☐ Railway wagon ☐ Road vehicle ☐ Other ☐ Identification:		I.17. Transporter Name Approval number Address Postal code Member State	
I.18. Description of commodity		I.19. Commodity code (CN code)	
		I.20. Number/quantity	
I.21.		I.22. Number of packages	
I.23. Identification of container/seal number		I.24. Type of packaging	
I.25. Commodities certified for Breeding ☐ Registered equidae ☐ Slaughter ☐ Other ☐			
I.26. Transit through third country ☐ Third country ISO code Exit point Code Entry point BIP unit no.:		I.27. Transit through Member States ☐ Member State ISO code Member State ISO code Member State ISO code	
I.28. Export ☐ Third country ISO code Exit point Code		I.29. Estimated journey time	
I.30. Route plan Yes ☐ No ☐			
I.31. Identification of the commodities Species (Scientific name) Identification system			

EUROPEAN COMMUNITY **Registered equidae, equidae for breeding and production equidae for slaughter**

II.a. Certificate reference number	II.b. Local reference number

Part II: Certification

II. **Health information** (1)

I, the undersigned, certify that the animal/s described above meet/s the following requirements:

II.1. it/they has/have been examined today and show/s no clinical sign of disease;

II.2. it/they is/are not intended for slaughter under a national programme of contagious or infectious disease eradication;

either (2) [II.3. it/they does/do not come from the territory or part of the territory of a Member State, which is the subject of restrictions for reasons of African horse sickness;]

or (2) [II.3. it/they come/s from the territory or part of the territory of a Member State, which is the subject of restrictions for reasons of African horse sickness, have remained for at least 40 days prior to dispatch in the vector proved quarantine station of ..... and has/have undergone a test for the detection of antibodies to the African horse sickness virus as described in Annex IV to Directive 2009/156/EC carried out simultaneously on blood samples taken on two occasions with an interval of between 21 and 30 days on ..... *(insert date)* and during the 10 days prior to dispatch on ..... *(insert date);*

either (3) [with negative result in each case if it/they was/were not vaccinated against African horse sickness;]

or (2) [without increase in antibody count, if it/they was/were vaccinated against African horse sickness;]

either (3) [II.4. it/they is/are not vaccinated against African horse sickness;]

or (2) [II.4. it/they was/were vaccinated against African horse sickness on ..... *(insert date)*,

either (3) [at least two months prior to certification;]

or (2) [at least two months prior to entry into the quarantine station;]

II.5. it/they does/do not come from (a) holding(s) which was/were subject to prohibition order(s) for animal health reasons which laid down at least of one the following conditions:

either (3) [not all the animals on the holding of species susceptible to the diseases mentioned in points (a) to (g) hereinafter were slaughtered and the prohibition lasted at least for:

(a) in the case of equidae suspected of having contracted dourine,

either (2) [six months beginning on the date of the last actual or possible contact with a sick or infected with *Trypanosoma equiperdum* animal;]

or (2) [in the case of a stallion, until the animal is castrated;]

(b) in the case of glanders, six months beginning on the day on which the equidae suffering from the disease or subjected with positive result to a test for the detection of the causative pathogen *Burkholderia mallei* or antibodies to that pathogen, were killed and destroyed;

(c) in the case of equine encephalomyelitis of any type, six months beginning on the day on which the equidae suffering from the disease have been slaughtered, except in case of West Nile virus infection where the period of six months begins on the day the infected equidae died, have been removed from the holding or fully recovered;

EUROPEAN COMMUNITY **Registered equidae, equidae for breeding and production equidae for slaughter**

II. **Health information** (1)	II.a. Certificate reference number	II.b. Local reference number

(d) in the case of infectious anaemia, until the date on which, the infected animals having been slaughtered, the remaining animals have shown a negative reaction to a Coggins test carried out on blood samples collected on two occasions three months apart;

(e) in the case of vesicular stomatitis, six months from the last case;

(f) in the case of rabies, one month from the last case;

(g) in the case of anthrax, 15 days from the last case;]

or (2) [following cases of dourine, glanders, equine encephalomyelitis of all types, equine infectious anaemia, vesicular stomatitis, anthrax or rabies all animals on the holding of species susceptible to the disease in question were slaughtered or killed and the prohibition lasted for 30 days, or 15 days in the case of anthrax, beginning on the day on which, following the destruction of the animals, the disinfection of the premises was satisfactorily completed;]

II.6. to the best of my knowledge, it/they has/have not been in contact with equidae suffering from an infectious or contagious disease in the 15 days prior to this declaration;

II.7. at the time of the inspection it/they was/were fit to be transported on the intended journey in accordance with the provisions of Regulation (EC) No 1/2005 (3).

Notes

Part I

Box I.6: shall correspond to the CITES permit number in case of equidae listed in the Washington Convention on protected species and products thereof.

Box I.16: Registration number (railway wagons or container and lorries), flight number (aircraft) or name (ship).

Box I.19: Use the appropriate Harmonised System (HS) code of the World Customs Organisation: 01.01.01 or 01.01.06.19

Box I.31: Species: horse, ass, mule, hinny, zebra (including their crossings).

Identification system: Until 31 December 2009 shall correspond to an identification number as described in Article 2 of Commission Decision 2000/68/EC, and as of 1 January 2010 to the unique life number as described in Article 2(2)(d) of and Section 1(A)(4) of Annex I to Commission Regulation (EC) No 504/2008.

Part II

(1) The information in points II.1. to II.6. is not required where there is a bilateral agreement in accordance with Article 6 of Directive 2009/156/EC.

(2) Delete whichever does not apply.

(3) This statement does not exempt transporters from their obligations in accordance with Community provisions in force in particular regarding the fitness of animals to be transported.

— This certificate is valid for 10 days.

— The colour of the stamp and signature must be different from that of the other particulars in the certificate.

Official veterinarian or official inspector

Name (in Capital): Qualification and title

Local Veterinary Unit: N° of the related LVU

Date: Signature:

Stamp

[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

The serological method described hereinafter are enzyme-linked immunosorbent assays (ELISA) based on point 2 of Section B in Chapter 2.5.1 of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Edition 2016 as adopted by the World Assembly of Delegates of the OIE in May 2012.

The VP7 viral protein is an immuno-dominant major antigen of the African horse sickness virus (AHSV) and is conserved across the nine AHSV serotypes. Recombinant AHSV-VP7 proteins have been shown to be stable and innocuous and suitable to be used as antigens in ELISA procedures for determination of AHSV antibodies with a high degree of sensitivity and specificity (Laviada et al., 1992b[6]; Maree and Paweska, 2005). The indirect ELISA and the blocking ELISA are the two AHS-VP7 ELISA tests suitable for serological diagnosis of African horse sickness (AHS).

1. **Indirect ELISA for the detection of antibodies to African horse sickness virus (AHSV)**

The conjugate used in this method is a horseradish peroxidase anti-horse gamma-globulin reacting with the serum of horses, mules and donkeys. The method described by Maree & Paweska (2005)[7] uses protein G as conjugate that also reacts with zebra serum.

The antigen may be provided by the Centro de Investigación en Sanidad Animal (CISA), Spain, within 4 to 6 months of request.

1.1. *Test procedure*

1.1.1. Solid phase

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

1.1.1.2. Wash the plates five times with distilled water containing 0,01 % (v/v) Tween 20 (washing solution). Gently tap the plates onto absorbent material to remove any residual wash.

1.1.1.3. Block the plates with phosphate buffered saline (PBS) pH 7,2 + 5 % (w/v) skimmed milk (Nestlé Dry Skim Milk™), 200 µl/well, for 1 hour at 37 °C.

1.1.1.4. Remove the blocking solution and gently tap the plates onto absorbent material.

1.1.2. Test samples

1.1.2.1. Serum samples to be tested, and positive and negative control sera, are diluted 1 in 25 in PBS + 5 % (w/v) skimmed milk + 0,05 % (v/v) Tween 20, 100 µl per well. Incubate for 1 hour at 37 °C.

For titration, make a twofold dilution series from 1 in 25 (100 µl/well), one serum per plate column, and do the same with positive and negative controls. Incubate for 1 hour at 37 °C.

1.1.2.2. Wash the plates five times with distilled water containing 0,01 % (v/v) Tween 20 (washing solution). Gently tap the plates onto absorbent material to remove any residual wash.

1.1.3. Conjugate

1.1.3.1. Dispense 100 µl/well of horseradish-peroxidase (HRP) -conjugated anti-horse gamma-globulin diluted in PBS + 5 % milk + 0,05 % Tween 20, pH 7,2. Incubate for 1 hour at 37 °C.

1.1.3.2. Wash the plates five times with distilled water containing 0,01 % (v/v) Tween 20 (washing solution). Gently tap the plates onto absorbent material to remove any residual wash.

1.1.4. Chromogen/Substrate

1.1.4.1. Add 200 µl/well of chromogen/substrate solution (10 ml of 80,6 mM DMAB (dimethyl aminobenzaldehyde) + 10 ml of 1,56 mM MBTH (3-methyl-2-benzo-thiazoline hydrazone hydrochlorid) + 5 µl H_2O_2).

Colour development is stopped by adding 50 µl of 3N H_2SO_4 after approximately 5 to 10 minutes (before the negative control begins to be coloured).

Other chromogens such as ABTS (2,2′-Azino-bis-[3-ethylbenzothiazoline-6-sulphonic acid]), TMB (tetramethyl benzidine), or OPD (ortho-phenyldiamine) can also be used.

1.1.4.2. Read the plates at 600 nm (or 620 nm).

1.2. *Interpretation of the results*

1.2.1. Calculate the cut-off value by adding 0,06 to the value of the negative control (0,06 is the standard deviation derived with a group of 30 negative sera).

1.2.2. Test samples giving absorbance values lower than the cut-off are regarded as negative.

1.2.3. Test samples giving absorbance values greater than the cut-off + 0,15 are regarded as positive.

1.2.4. Test samples giving intermediate absorbance values are considered to be inconclusive and a second technique must be employed to confirm the result.

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:

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

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.

PART B

Identification of the agent

Real-time Reverse-Transcription Polymerase Chain Reaction (rRT-PCR)

Agent identification tests based on nucleic acid methods must detect reference strains from the nine virus serotypes of the AHSV.

The method described in point 2.1 is based on point 1.2 of Section B in Chapter 2.5.1 of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Edition 2016 as adopted by the World Assembly of Delegates of the OIE in May 2012.

Any RT-PCR detection method used for the testing of samples, either blood or spleen, in the context of Directive 2009/156/EC must perform equal to or exceed the sensitivity of the methodologies described in point 2.

Inactivated virus of serotypes 1 to 9 reference strains may be obtained from the European Union Reference Laboratory or the OIE Reference Laboratory for African horse sickness, Algete, Spain.

1. **Extraction of viral RNA**

To assure a good reaction it is necessary to extract from the sample an AHSV RNA of high quality. The extraction of nucleic acids from clinical samples can be performed by a variety of in-house and commercially available methods.

Commercial kits use different approaches for RNA isolation. Most are based on one of the following procedures:

— Phenol-chloroform extraction of nucleic acids;

— Adsorption of nucleic acids to filter system;

— Adsorption of nucleic acids to magnetic beads system.

An example of an in-house RNA extraction is given below:

1.1. 1 g of tissue sample is homogenised in 1 ml of denaturing solution (4 M guanidium thiocyanate, 25 mM sodium citrate, 0,1 M 2-mercaptoethanol, 0,5 % sarcosyl).

1.2. After centrifugation, 1 µg of yeast RNA, 0,1 ml of 2 M sodium acetate pH 4, 1 ml of phenol and 0,2 ml of chloroform/isoamyl alcohol mixture (49/1) are added to the supernatant.

1.3. The suspension is vigorously shaken and cooled on ice for 15 minutes.

1.4. After centrifugation, the RNA present in the aqueous phase is phenol extracted, ethanol precipitated and resuspended in sterile water.

2. **Real-time RT-PCR Procedure**

2.1. *Group-specific real-time RT-PCR by Agüero et al., 2008*[8]

This group-specific real-time RT-PCR targets VP7 of the AHSV and is able to detect all known AHSV serotypes and strains currently circulating. It has been employed with very good results by the participating national reference laboratories of the European Union Member States in the proficiency tests annually organised by the European Union Reference Laboratory for the period 2009-2015. Moreover, in an international ring trial organised in 2015 in the framework of the OIE reference laboratories network this protocol was ranked very high amongst others.

Primer and probe sequences for the detection of AHSV species viruses:

—forward Primer 5′-CCA-GTA-GGC-CAG-ATC-AAC-AG-3′
—reverse Primer 5′-CTA-ATG-AAA-GCG-GTG-ACC-GT-3′
—MGB-TaqMan probe 5′-FAM-GCT-AGC-AGC-CTA-CCA-CTA-MGB-3′

2.1.1. Primer stock concentration is diluted to a working concentration of 8 µM (‘primer working stock 8 µM’) whereas probe is diluted to a working concentration of 50 µM (‘probe working stock 50 µM’). A test plate layout should be designed and loaded into the real time PCR machine software. Using the layout as a guide, 2,5 µl of each primer working stock 8 µM is added to each well that will contain RNA samples, positive and/or negative controls (final concentration of the primer will be 1 µM in the 20 µl RT-PCR mix). The plate is held on ice.

2.1.2. 2 µl of isolated RNA (test samples and positive control), or 2 µl of RNAse-free water in negative reaction controls, is mixed with forward and reverse primers. This mixture is denatured by heating at 95 °C for 5 minutes, followed by rapid cooling on ice for at least 5 minutes.

2.1.3. An appropriate volume of real time one-step RT-PCR master mix for the number of samples to be tested is prepared following manufacturer's instructions. 0,1 µl of probe working stock 50 µM is added to each well containing RNA samples (final concentration of the probe will be 0,25 µM in each well containing RNA samples). 13 µl of real time one-step RT-PCR master mix is distributed in each well on the PCR plate containing the denatured primers and RNA.

2.1.4. The plate is placed in a real time thermal cycler programmed for reverse transcription and cDNA amplification/fluorescence detection. Amplification conditions consist of a first reverse-transcription step at 48 °C for 25 minutes, followed by 10 minutes at 95 °C (‘hot start’) and 40 cycles of 15 seconds at 95 °C, 35 seconds at 55 °C and 30 seconds at 72 °C (or 40 cycles at 97 °C for 2 seconds and 55 °C for 30 seconds if reagents and thermocycler allowing fast reactions are used). Fluorescence data are acquired at the end of the 55 °C step.

2.1.5. The assay is considered not valid if atypical amplification curves are obtained, and must be repeated.

Samples are considered positives, if the Ct value (cycle number at which the fluorescence generated within a reaction crosses the fluorescence threshold) is lower than or equal to the defined Ct threshold (35) within 40 PCR cycles (Ct ≤ 35).

Samples are considered inconclusive, if the Ct value is higher than the defined Ct threshold (35) within 40 PCR cycles (Ct > 35).

Samples are considered negative, if a horizontal amplification curve is obtained which does not cross the threshold line within 40 PCR cycles.

2.2. *Group-specific real-time RT-PCR by Guthrie et al., 2013*[9]

Real-time RT-PCR using fluorescence resonance energy transfer (FRET) probes to detect nucleic acid of AHSV.

The AHSV RT-PCR assay described was designed using sequences from a wide variety of currently circulating field strains of AHSV (Quan et al., 2010[10]). It also incorporates a proprietary synthetic external control assay to verify proper functioning of the assay components.

Kits for the one-step real-time PCR are available commercially. Below are some basic steps as described by Guthrie et al. (2013), which can be modified depending upon local/case-specific requirements, kits used and equipment available.

Primer and probe sequences for the detection of AHSV species viruses:

—forward Primer	5′-AGA-GCT-CTT-GTG-CTA-GCA-GCC-T-3′
—reverse Primer	5′-GAA-CCG-ACG-CGA-CAC-TAA-TGA-3′
—MGB-TaqMan probe	5′-FAM-TGC-ACG-GTC-ACC-GCT-MGB-3′

2.2.1. Primer and probe mix stock solutions are made up in a 25× concentration at 5 µM for the forward and reverse primers and 3 µM for the probe. A test plate layout should be designed and loaded into the real-time PCR machine software. Using the layout as a guide, 5 µl of RNA samples, including test samples and positive and negative controls, are added to appropriate wells of the plate following the layout.

2.2.2. The RNA is denatured by heating at 95 °C for 5 minutes, followed by rapid cooling on ice for at least 3 minutes.

2.2.3. An appropriate volume of real-time one-step RT-PCR master mix for the number of samples to be tested is prepared, following the manufacturer's instructions. 1 µl of 25× primer probe mix stock solution (from point 2.2.1 above) is included in the master mix to give a final concentration in each well of 200 nM for each primer and 120 nM of the probe. 20 µl of the master mix is distributed in each well on the PCR plate containing the denatured RNA.

2.2.4. The plate is placed in a real-time thermal cycler programmed for reverse transcription and cDNA amplification/fluorescence detection as suggested by the manufacturers. Amplification conditions consist of, for example, a first reverse-transcription step at 48 °C for 10 minutes, followed by 10 minutes at 95 °C and 40 cycles of 15 seconds at 95 °C and 45 seconds at 60 °C.

2.2.5. Samples are considered positives, if the normalised fluorescence for the AHSV RT-PCR assay exceeds a 0,1 threshold within 36 PCR cycles in all replicates of a sample.

Samples are considered inconclusive, if the normalised fluorescence for the AHSV RT-PCR assay exceeds a 0,1 threshold between 36 and 40 PCR cycles in any replicate of a sample.

Samples are considered negative, if the normalised fluorescence for the AHSV RT-PCR assay did not exceed a 0,1 threshold within 40 PCR cycles in all replicates of a sample and if the normalised fluorescence for the proprietary synthetic external control assay exceeded a 0,1 threshold within 33 PCR cycles.**]**

ANNEX V

PART A

REPEALED DIRECTIVE WITH LIST OF ITS SUCCESSIVE AMENDMENTS

(referred to in Article 22)

Council Directive 90/426/EEC (OJ L 224, 18.8.1990, p. 42).	
Council Directive 90/425/EEC (OJ L 224, 18.8.1990, p. 29).	only Article 15(3)
Council Directive 91/496/EEC (OJ L 268, 24.9.1991, p. 56).	only as regards the reference to Directive 90/426/EEC in Article 26(2)
Commission Decision 92/130/EEC (OJ L 47, 22.2.1992, p. 26).	
Council Directive 92/36/EEC (OJ L 157, 10.6.1992, p. 28).	only Article 1
1994 Act of Accession, Annex I, Point V.E.I.A.3 (OJ C 241, 29.8.1994, p. 132).	
Commission Decision 2001/298/EC (OJ L 102, 12.4.2001, p. 63).	only as regards the reference to Directive 90/426/EEC in Article 1(1), and Annex I, pt. 2
Commission Decision 2002/160/EC (OJ L 53, 23.2.2002, p. 37).	
Council Regulation (EC) No 806/2003 (OJ L 122, 16.5.2003, p. 1).	only Annex III, point 10
2003 Act of Accession, Annex II, Point 6.B.I.16 (OJ L 236, 23.9.2003, p. 381).	
Council Directive 2004/68/EC (OJ L 139, 30.4.2004, p. 321).	only Article 15
Council Directive 2006/104/EC (OJ L 363, 20.12.2006, p. 352).	only Annex, point I.2.

Council Directive 2008/73/EC (OJ L 219, 14.8.2008, p. 40).	only Article 7

PART B

LIST OF TIME-LIMITS FOR TRANSPOSITION INTO NATIONAL LAW

(referred to in Article 22)

Directive	**Time-limit for transposition**
90/426/EEC	1 January 1992
90/425/EEC	1 July 1992
91/496/EEC	1 July 1992
92/36/EEC	31 December 1992
2004/68/EC	19 November 2005
2006/104/EC	1 January 2007
2008/73/EC	1 January 2010

ANNEX VI

CORRELATION TABLE

Directive 90/426/EEC	**This Directive**
Article 1	Article 1
Article 2(a) and (b)	Article 2(a) and (b)
Article 2(c)	Article 2(c)(i) and (ii)
Article 2(d) to (i)	Article 2(d) to (i)
Article 3	Article 3
Article 4(1), (2) and (3)	Article 4(1), (2) and (3)
Article 4(4)(i) and (ii)	Article 4(4)(a) and (b)
Article 4(5)(a), first to sixth indents	Article 4(5)(a)(i) to (vi)
Article 4(5)(b)	Article 4(5)(b)
Article 4(6), first subparagraph, first to eighth indents	Article 4(6), first subparagraph, (a) to (h)
Article 4(6), second and third subparagraphs	Article 4(6), second and third subparagraphs
Article 5(1)	Article 5(1)
Article 5(2)(a)	Article 5(2), first subparagraph, (a) and (b)
Article 5(2)(b)	Article 5(2), second subparagraph, (a) and (b)

Article 5(2)(c)	Article 5(3)
Article 5(2)(d)	Article 5(4)
Article 5(3)(a) and (b)	Article 5(5)(a) and (b)
Article 5(3)(c), first and second indents	Article 5(5)(c), first subparagraph, (i) and (ii)
Article 5(3)(c), second indent, last sentence	Article 5(5)(c), second subparagraph
Article 5(3)(d) and (e)	Article 5(5)(d) and (e)
Article 6	Article 6
Article 7	Article 7
Article 8(1), first subparagraph, first and second indents	Article 8(1)(a) and (b)
Article 8(1), second subparagraph	Article 8(2)
Article 8(2)	Article 8(3)
Article 9	Article 9
Article 10	Article 10
Article 11(1)	Article 11
Article 11(2)	—
Article 12	Article 12
Article 13	Article 13
Article 14	Article 14
Article 15	Article 15
Article 16(1)(a) to (f)	Article 16(1)(a) to (f)
Article 16(1), final sentence	—
Article 16(2)	Article 16(2)
Article 17	Article 18
Article 18	Article 17
Article 19(i) to (iv)	Article 19(a) to (d)
Article 22	—
Article 23	Article 20
Article 24(1) and (2)	Article 21(1) and (2)
Article 24(3)	—
Article 25(1) and (2)	Article 21(1) and (3)
Article 26	—
Article 27	—
—	Article 22

—	Article 23
Article 28	Article 24
Annex A	Annex I
Annex B	Annex II
Annex C	Annex III
Annex D	Annex IV
—	Annex V
—	Annex VI

(**1**) This attestation is not required where there is a bilateral agreement in accordance with Article 6 of Directive 2009/156/EC.

(**2**) Valid for 10 days.

(**3**) Delete whichever does not apply.

(**4**) The vaccination date must be entered in the passport.

(**5**) This statement does not exempt transporters from their obligations in accordance with Community provisions in force in particular regarding the fitness of animals to be transported.

(**6**) **[**[F1]Laviada M.D., Roy P. and Sanchez-Vizcaino J.M (1992b). Adaptation and evaluation of an indirect ELISA and immunoblotting test for African horse sickness antibody detection. In: Bluetongue, African Horse Sickness and Related Orbiviruses: Proceedings of the Second International Symposium. Walton T.E. & Osburn B.l., Eds. CRC Press, Boca Raton, Florida, USA, 646-650.**]**

(**7**) **[**[F1]Maree S. and Paweska J.T. (2005). Preparation of recombinant African horse sickness virus VP7 antigen via a simple method and validation of a VP7-based indirect ELISA for the detection of group-specific IgG antibodies in horse sera. J. Virol. Methods, 125 (1), 55-65.**]**

(**8**) **[**[F1]Agüero M., Gomez-Tejedor C., Angeles Cubillo M., Rubio C., Romero E. and Jimenez-Clavero A. (2008). Real-time fluorogenic reverse transcription polymerase chain reaction assay for detection of African horse sickness virus. J. Vet. Diagn. Invest., 20, 325-328.**]**

(**9**) **[**[F1]Guthrie AJ, MacLachlan NJ, Joone C, Lourens CW, Weyer CT, Quan M, Monyai MS, Gardner IA. Diagnostic accuracy of a duplex real-time reverse transcription quantitative PCR assay for detection of African horse sickness virus. Journal of Virological Methods. 2013;189(1):30-5.**]**

(**10**) **[**[F1]Quan, M., Lourens, C.W., MacLachlan, N.J., Gardner, I.A., Guthrie, A.J., 2010. Development and optimisation of a duplex real-time reverse transcription quantitative PCR assay targeting the VP7 and NS2 genes of African horse sickness virus. J. Virol. Methods 167, 45-52.**]**

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).