

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)

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)

THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty establishing the European Community, and in particular Article 37 thereof,

Having regard to the proposal from the Commission,

Having regard to the Opinion of the European Parliament⁽¹⁾,

Whereas:

- (1) Council Directive 90/426/EEC of 26 June 1990 on animal health conditions governing the movement and import from third countries of equidae⁽²⁾ has been substantially amended several times⁽³⁾. In the interests of clarity and rationality the said Directive should be codified.
- (2) Equidae, being live animals, are included in the list of products in Annex I to the Treaty.
- (3) In order to ensure the rational development of equidae production, thereby increasing productivity in that sector, rules governing the movement of equidae between Member States should be laid down at Community level.
- (4) The breeding and rearing of equidae, and in particular of horses, is generally included in the farming sector. It constitutes a source of income for part of the farming population.
- (5) Disparities as regards animal health conditions in the Member States should be eliminated in order to encourage intra-Community trade in equidae.
- (6) In order to encourage the harmonious development of intra-Community trade, a Community system should be provided for to govern imports from third countries.
- (7) The conditions for the movement on national territory of registered equidae bearing an identification document should also be regulated.
- (8) In order to be the subject of trade, equidae should satisfy certain animal health requirements, so as to avoid the spreading of infectious or contagious diseases. It

appears in particular appropriate to provide for a possible regionalisation of restrictive measures.

- (9) The transport conditions should be laid down for the same reasons, taking into account the animal welfare conditions laid down in Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations⁽⁴⁾.
- (10) To ensure that those requirements are satisfied, provision should be made for the issue by an official veterinarian of a health certificate to accompany the equidae to their place of destination.
- (11) The organisation of and the follow-up to the checks to be carried out by the Member State of destination and the safeguard measures to be implemented have been laid down in Council Directive 90/425/EEC of 26 June 1990 concerning veterinary and zootechnical checks applicable in intra-Community trade in certain live animals and products with a view to the completion of the internal market⁽⁵⁾.
- (12) Provision should be made for the possibility of checks by the Commission. These checks should be carried out in cooperation with the competent national authorities.
- (13) Defining Community provisions applicable to imports from third countries requires a list to be drawn up of third countries or parts of third countries from which equidae may be imported.
- (14) The choice of those countries should be based on criteria of a general nature such as the state of health of the livestock, the organisation and powers of the veterinary services and the health regulations in force.
- (15) In addition, imports of equidae should not be authorised from countries infected with infectious or contagious animal diseases which present a risk to Community livestock or which have been free from such infection for too short a period. Such considerations are also valid for imports from third countries in which vaccination against such diseases is carried out.
- (16) The general conditions applicable to imports from third countries should be supplemented by special conditions drawn up on the basis of the health situation in each of them. The technical nature and the diversity of the criteria on which those special conditions depend require for their definition recourse to a flexible and rapid Community procedure in which the Commission and the Member States cooperate closely.
- (17) The presentation of a common standard form of certificate upon import of equidae constitutes an effective means of verifying that the Community rules are being applied. Such rules may include special provisions which may vary according to the third country concerned, and this should be taken into account in drawing up the standard forms of certificates.
- (18) Veterinary experts of the Commission and of the Member States, appointed by the Commission, should be responsible for verifying that the requirements of this Directive are observed, particularly in third countries.

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- (19) The checks carried out upon importation should cover the origin and the state of health of the equidae.
- (20) The measures necessary for the implementation of this Directive should be adopted in accordance with Council Decision 1999/468/EC of 28 June 1999 laying down the procedures for the exercise of implementing powers conferred on the Commission⁽⁶⁾.
- (21) This Directive is without prejudice to the obligations of the Member States relating to the time-limits for transposition into national law of the Directives set out in Annex V, Part B,

HAS ADOPTED THIS DIRECTIVE:

CHAPTER I

GENERAL PROVISIONS

Article 1

This Directive lays down animal health conditions for the movement between Member States and importation from third countries of live equidae.

Article 2

For the purposes of this Directive the following definitions shall apply:

- (a) 'holding' means an agricultural or training establishment, a stable or, generally speaking, any premises or facilities in which equidae are habitually kept or bred, for whatever use;
- (b) 'equidae' means wild or domesticated animals of the equine (including zebras) or asinine species or the offspring of crossings of those species;
- (c) 'registered equidae' means any equidae registered as defined in Council Directive 90/427/EEC of 26 June 1990 on the zootechnical and genealogical conditions governing intra-Community trade in equidae⁽⁷⁾, identified by means of an identification document issued by:
 - (i) the breeding authority or any other competent authority of the country where the animal originated which manages the studbook or register for that breed of animal; or
 - (ii) any international association or organisation which manages horses for competition or racing;
- (d) 'equidae for slaughter' means equidae intended to be transported either directly or after transit through an approved marshalling centre, referred to in Article 7, to the slaughterhouse for slaughter;
- (e) 'equidae for breeding and production' means equidae other than those mentioned in (c) and (d);
- (f) 'Member State or third country free from African horse sickness' means any Member State or third country in which there has been no clinical, serological (in unvaccinated equidae) or epidemiological evidence of African horse sickness on the territory

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concerned in the previous two years and in which there have been no vaccinations against the disease during the previous 12 months;

- (g) ‘compulsorily notifiable diseases’ means the diseases listed in Annex I;
- (h) ‘official veterinarian’ means the veterinarian designated by the competent central authority of a Member State or of a third country;
- (i) ‘temporary admission’ means the status of registered equidae originating in a third country and admitted into Community territory for a period of less than 90 days to be fixed in accordance with the procedure referred to in Article 21(2), depending on the health situation in the country of origin.

CHAPTER II

RULES FOR THE MOVEMENT OF EQUIDAE BETWEEN MEMBER STATES

Article 3

Member States shall authorise the movement of registered equidae in their territory or send equidae to another Member State only where they satisfy the conditions laid down in Articles 4 and 5.

However, the competent authorities in Member States of destination may grant general or limited exemption in respect of movement of equidae which:

- are being ridden or taken, for sporting or recreational purposes, along roads situated near internal borders of the Community,
- are taking part in cultural or similar events or in activities organised by authorised local bodies situated near internal borders of the Community,
- are intended solely for temporary pasturing or work near internal borders of the Community,

Member States making use of such authorisation shall inform the Commission of the content of the exemptions granted.

Article 4

1 Equidae must show no clinical sign of disease at inspection. Inspection must be carried out in the 48 hours prior to their embarkation or loading. In the case of registered equidae, however, this inspection shall, without prejudice to Article 6, be required for intra-Community trade only.

2 Without prejudice to the requirements of paragraph 5 regarding compulsorily notifiable diseases, the official veterinarian must, at the time of inspection, be satisfied that there are no grounds — in particular on the basis of declarations by the owner or breeder — for concluding that the equidae have been in contact with equidae suffering from an infectious or contagious disease during the 15 days immediately preceding inspection.

3 The equidae must not be intended for slaughter under a national programme of infectious or contagious disease eradication.

4 The equidae must be identified in the following manner:

- a in the case of registered equidae, by means of an identification document, as provided for in Directive 90/427/EEC, which must certify in particular that paragraphs 5 and 6 of this Article and Article 5 of this Directive have been complied with.

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The official veterinarian must suspend the validity of the identification document for the period of the prohibitions provided for in paragraph 5 of this Article or in Article 5 of this Directive. The identification document must, following the slaughter of the registered horse, be returned to the authority which issued it. The procedure for the implementation of this point shall be adopted in accordance with the procedure referred to in Article 21(2);

- b for equidae for breeding and production, by the method established in accordance with the procedure referred to in Article 21(2).

5 In addition to the requirements laid down in Article 5, the equidae must not come from a holding which has been the subject of one of the following prohibition orders:

- a if all the animals of species susceptible to the disease located on the holding have not been slaughtered, the period of prohibition concerning the holding of origin must be at least:
 - (i) 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;
 - (ii) 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;
 - (iii) 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;
 - (iv) six months from the last recorded case, in the case of vesicular stomatitis;
 - (v) one month from the last recorded case, in the case of rabies;
 - (vi) 15 days from the last recorded case, in the case of anthrax;
- b if all the animals of species susceptible to the disease located on the holding have been slaughtered and the premises disinfected, the period of prohibition shall be 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.

The competent authorities may derogate from these prohibition orders for hippodromes and racecourses, and shall notify the Commission of the nature of any derogations granted.

6 [F¹Where a Member State draws up or has drawn up a voluntary or compulsory control programme for a disease to which equidae are susceptible, it may present the programme to the Commission, within six months from 4 July 1990 for Belgium, Denmark, Germany, Ireland, Greece, Spain, France, Italy, Luxembourg, the Netherlands, Portugal and the United Kingdom, from 1 January 1995 for Austria, Finland and Sweden, from 1 May 2004 for the Czech Republic, Estonia, Cyprus, Latvia, Lithuania, Hungary, Malta, Poland, Slovenia and Slovakia, from 1 January 2007 for Bulgaria and Romania and from 1 July 2013 for Croatia, outlining in particular:]

- a the distribution of the disease on its territory;
- b the reasons for the programme, taking into consideration the significance of the disease and its cost/benefit advantages;
- c the geographical area in which the programme will be implemented;

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- d the status categories to be applied to establishments, the standards which must be attained for each species and the test procedures to be used;
- e the programme monitoring procedures;
- f the action to be taken if, for any reason, a holding loses its status;
- g the measures to be taken if the results of the tests carried out in accordance with the provisions of the programme are positive;
- h the non-discriminatory nature of trade in the territory of the Member State concerned with respect to intra-Community trade.

The Commission shall examine the programmes presented by the Member States. Where appropriate, it shall approve them in accordance with the procedure referred to in Article 21(2). Any additional guarantees, general or specific, which may be required in intra-Community trade may be defined in accordance with the same procedure. Such guarantees must not exceed those required by the Member State in its own territory.

Programmes submitted by Member States may be amended or supplemented in accordance with the procedure referred to in Article 21(3). Amendments or additions to programmes which have already been approved or to guarantees which have been defined in accordance with the second subparagraph may be approved under the same procedure.

Textual Amendments

- F1** Substituted by [Council Directive 2013/20/EU of 13 May 2013 adapting certain directives in the field of food safety, veterinary and phytosanitary policy, by reason of the accession of the Republic of Croatia.](#)

Article 5

1 A Member State which is not free from African horse sickness may dispatch equidae from that part of its territory which is considered to be infected within the meaning of paragraph 2 of this Article only under the conditions set out in paragraph 5.

2 A part of the territory of a Member State shall be considered to be infected with African horse sickness if:

- a clinical, serological (in unvaccinated animals) and/or epidemiological evidence has revealed the presence of African horse sickness in the past two years; or
- b vaccination against African horse sickness has been carried out in the past 12 months.

The part of the territory considered to be infected with African horse sickness shall comprise as a minimum:

- a a protection zone with a radius of at least 100 km around any centre of infection;
- b a surveillance zone of at least 50 km extending beyond the protection zone, in which no vaccination has been carried out in the last 12 months.

3 The control rules and the measures to combat African horse sickness relating to the territories and zones referred to in paragraph 2 and the relevant derogations are specified in Council Directive 92/35/EEC of 29 April 1992 laying down control rules and measures to combat African horse sickness⁽⁸⁾.

4 All vaccinated equidae found in the protection zone must be registered and marked in accordance with Article 6(1)(d) of Directive 92/35/EEC.

The identification document and/or health certificate shall carry a clear reference to such vaccination.

5 A Member State may dispatch from the territory referred to in the second subparagraph of paragraph 2 only equidae which meet the following requirements:

- a they must be dispatched only during certain periods of the year, having regard to the activity of vector insects, to be determined in accordance with the procedure referred to in Article 21(3);
- b they must show no clinical symptom of African horse sickness on the day of the inspection referred to in Article 4(1);
- c they must have undergone a test for African horse sickness as described in Annex IV, on two occasions, with an interval of between 21 and 30 days between the two tests, the second of which must have been carried out during the 10 days prior to dispatch either:
 - (i) with negative results, if they have not been vaccinated against African horse sickness; or
 - (ii) without having recorded an increase in the antibody count and without having undergone vaccination during the previous two months, if they have been vaccinated against African horse sickness.

In accordance with the procedure referred to in Article 21(2), and following the opinion of the European Food Safety Authority, other monitoring methods may be recognised;

- d they must have been kept in a quarantine station for a minimum period of 40 days prior to dispatch;
- e they must have been protected from vector insects during the period of quarantine and during transportation from the quarantine station to the place of dispatch.

Article 6

Member States which implement an alternative control system providing guarantees equivalent to those laid down in Article 4(5) as regards movements within their territory of equidae may grant one another derogations from the provisions of the second sentence of Article 4(1) and Article 8(1)(b) on a reciprocal basis.

They shall notify the Commission thereof.

Article 7

1 Equidae must be transported, as soon as possible, from the holding of origin either directly or via an approved marshalling centre, as defined as ‘assembly centre’ in Article 2(2) (o) of Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine⁽⁹⁾, to the place of destination in vehicles or containers which have been regularly cleansed and disinfected with a disinfectant at intervals to be fixed by the Member State of dispatch. The vehicles must be designed in such a way that equidae droppings, litter or fodder cannot escape from the vehicle during transportation. Without prejudice to Regulation (EC) No 1/2005, transportation must be effected in such a way that the health and well-being of the equidae can be protected effectively.

2 The Member State of destination may, on a general or restricted basis, grant a derogation from some of the requirements of Article 4(5) for any animal bearing a special mark indicating that it is scheduled for slaughter, provided that the health certificate in accordance with Annex III mentions such derogation.

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Where such derogation is granted, equidae for slaughter must be transported directly to the designated slaughterhouse and be slaughtered within five days of arrival at the slaughterhouse.

3 The official veterinarian must record the identification number or identification document number of the slaughtered animal and forward to the competent authority of the place of dispatch, at the latter's request, an attestation to the effect that the animal has been slaughtered.

Article 8

1 Member States shall ensure that:

- a registered equidae which leave their holdings are accompanied by the identification document laid down in Article 4(4)(a) together, if they are intended for intra-Community trade, with the health attestation provided for in Annex II;
- b equidae for breeding, production and slaughter are, during their transportation, accompanied by a health certificate complying with Annex III.

2 The health certificate, or in the case of registered equidae the health attestation, must, without prejudice to Article 6, be drawn up during the 48 hours preceding their embarkation or else no later than the last working day prior to it, in at least one of the official languages of the Member States of dispatch and destination. The duration of validity of the health certificate or health attestation shall be 10 days. The health certificate or health attestation must consist of a single sheet.

3 For the movement between Member States, equidae other than registered equidae may be covered by a single health certificate per consignment rather than by the individual health certificate referred to in paragraph 1, point (b).

Article 9

The rules laid down in Directive 90/425/EEC shall apply in particular to checks at origin, to the organisation of, and follow-up to, the checks to be carried out by the Member State of destination, and to the safeguard measures to be implemented.

Article 10

Veterinary experts from the Commission may, to the extent necessary to ensure uniform application of this Directive and in cooperation with the competent national authorities, carry out on-the-spot inspections. The Commission shall inform the Member States of the outcome of such inspections.

The Member States in whose territory an inspection is carried out shall give the experts all the assistance necessary to carry out their task.

General arrangements for the application of this Article shall be adopted in accordance with the procedure referred to in Article 21(2).

CHAPTER III

RULES FOR IMPORTATION OF EQUIDAE FROM THIRD COUNTRIES

Article 11

Equidae imported into the Community must satisfy the conditions laid down in Articles 12 to 16.

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Article 12

1 The importation of equidae into the Community shall only be authorised from third countries that appear on a list to be drawn up or amended in accordance with the procedure referred to in Article 21(2).

Taking into account the health situation and the guarantees provided by the third country for equidae, it may be decided in accordance with the procedure referred to in Article 21(2) that the authorisation provided for in the first subparagraph of this paragraph shall apply to the whole territory of the third country or to only part of its territory.

For that purpose and on the basis of the relevant international standards, account shall be taken of how the third country applies and implements those standards, in particular the principle of regionalisation, within its own territory and in relation to its sanitary requirements for importation from other third countries and from the Community.

2 When the list provided for in paragraph 1 is drawn up or amended, particular account shall be taken of:

- a the health status of the equidae, other domestic animals and wildlife in the third country, with particular regard to exotic animal diseases and any aspects of the general health and the environmental situation in the third country which may pose a risk to the health and environmental status of the Community;
- b the legislation of the third country in relation to animal health and welfare;
- c the organisation of the competent veterinary authority and its inspection services, the powers of those services, the supervision to which they are subject, and the means at their disposal, including staff and laboratory capacity, to apply national legislation effectively;
- d the assurances which the competent veterinary authority of the third country can give regarding compliance or equivalence with the relevant animal health conditions applicable in the Community;
- e whether the third country is a member of the World Organisation for Animal Health (OIE) and the regularity and rapidity of the information supplied by the third country relating to the existence of infectious or contagious diseases of equidae in its territory, in particular those diseases listed by the OIE and in Annex I to this Directive;
- f the guarantees given by the third country to directly inform the Commission and the Member States:
 - (i) within 24 hours, of the confirmation of the occurrence of infectious diseases of equidae listed in Annex I and of any change in the vaccination policy concerning such diseases;
 - (ii) within an appropriate period, of any proposed changes in the national sanitary rules concerning equidae, in particular regarding the importation of equidae;
 - (iii) at regular intervals, of the animal health status of its territory concerning equidae;
- g any experience of previous imports of live equidae from the third country and the results of any import controls carried out;
- h the results of Community inspections and/or audits carried out in the third country, in particular the results of the assessment of the competent authorities or, where the Commission so requests, the report submitted by the competent authorities on the inspections which they have carried out;

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- i the rules on the prevention and control of infectious or contagious animal diseases in force in the third country and their implementation, including rules on importation of equidae from other third countries.

3 The Commission shall arrange for up-to-date versions of the list drawn up or amended as provided for in paragraph 1 to be made available to the public.

The list may be combined with other lists drawn up for animal and public health purposes and may also include models of health certificates.

4 Special import conditions for each third country or group of third countries, having regard to the animal health situation concerning equidae in the third country or countries concerned shall be established in accordance with the procedure referred to in Article 21(2).

5 Detailed rules for the application of paragraphs 1 to 4 and criteria for including third countries or parts of third countries in the list provided for in paragraph 1 may be adopted in accordance with the procedure referred to in Article 21(2).

Article 13

1 The equidae must come from third countries which:

- a are free from African horse sickness;
- b have been free for two years from Venezuelan equine encephalomyelitis (VEE);
- c have been free for six months from dourine and glanders.

2 In accordance with the procedure referred to in Article 21(2) it may be decided:

- a that the provisions of paragraph 1 of this Article shall apply to only part of the territory of a third country.

In the event that the African horse sickness requirements apply on a regional basis, at the very least the measures laid down in Article 5(2) and (5) must be complied with;

- b to require additional guarantees for diseases alien to the Community.

Article 14

Before the day of loading for transportation to the Member State of destination, the equidae must have remained without interruption in the territory or part of the territory of a third country or, in the event of regionalisation, in the part of the territory defined pursuant to Article 13(2)(a) for a period to be determined in the decisions to be adopted pursuant to Article 15.

They must come from a holding placed under veterinary supervision.

Article 15

Importation of equidae from the territory of a third country or part thereof as defined in accordance with Article 13(2)(a) on the list drawn up in accordance with Article 12(1) shall be authorised only if the equidae, over and above the requirements of Article 13:

- (a) comply with the animal health requirements adopted, with reference to the species in question, the categories of equidae, in accordance with the procedure referred to in Article 21(2) for importation of equidae from that country.

The reference basis for fixing those animal health requirements shall be the standards laid down in Articles 4 and 5; and

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- (b) in the case of a third country not free of vesicular stomatitis or viral arteritis for at least six months, the equidae must meet the following requirements:
- (i) they must come from a holding which has been free of vesicular stomatitis for at least six months and they must have reacted negatively to a serological test prior to dispatch;
 - (ii) in the case of viral arteritis, male equidae must, notwithstanding Article 19(b), have reacted negatively to a serological test or to a virus isolation test or to any other test recognised in accordance with the procedure referred to in Article 21(2) which would guarantee freedom from the virus.

In accordance with the procedure referred to in Article 21(2), and following the opinion of the European Food Safety Authority, the categories of male equidae to which this requirement shall apply may be defined.

Article 16

1 The equidae must be identified in accordance with Article 4(4) and accompanied by a health certificate drawn up by an official veterinarian of the exporting third country. This health certificate must:

- a be issued on the day of loading of the animals for dispatch to the Member State of destination or, in the case of registered horses, on the last working day before embarkation;
- b be drawn up in at least one of the official languages of the Member State of destination and one of those of the Member State in which the import inspection is carried out;
- c accompany the animals in the original;
- d attest that the animals satisfy the requirements of this Directive and those laid down pursuant to this Directive with regard to importation from third countries;
- e consist of a single sheet;
- f be made out for a single consignee or, in the case of animals for slaughter, for a consignment, provided the animals are properly marked and identified.

Member States shall inform the Commission if they make use of this option.

2 The health certificate must be drawn up on a form complying with a model established in accordance with the procedure referred to in Article 21(2).

Article 17

1 Immediately upon arrival in the Member State of destination, equidae for slaughter shall be taken to a slaughterhouse, either directly or after transition through an approved marshalling centre, as referred to in Article 7, and, in accordance with animal health requirements, be slaughtered within a period specified in the decisions to be adopted pursuant to Article 15.

2 Without prejudice to any special conditions which may be adopted in accordance with the procedure referred to in Article 21(2), the competent authority of the Member State of destination may, on animal health grounds, designate the slaughterhouse to which such equidae must be taken.

Article 18

Checks shall be carried out on the spot by veterinary experts of the Member States and the Commission to verify whether the provisions of this Directive, and in particular those of Article 12(2), are being applied in practice.

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Should checks carried out within the terms of this Article bring to light serious facts as against an approved holding, the Commission shall immediately inform the Member States and forthwith adopt a decision provisionally suspending the approval. The final decision shall be taken in accordance with the procedure referred to in Article 21(3).

The experts from the Member States who are to be entrusted with those checks shall be appointed by the Commission, acting on a proposal from the Member States.

Those checks shall be made on behalf of the Community, which shall bear the cost of any expenditure incurred in this connection.

The frequency of and the procedure for those checks shall be determined in accordance with the procedure referred to in Article 21(2).

Article 19

In accordance with the procedure referred to in Article 21(2):

- (a) it may be decided that importation from a third country or part of a third country is to be confined to particular species or categories of equidae;
- (b) notwithstanding Article 15, the special conditions for the temporary entry into Community territory of registered equidae or equidae intended for special uses or their re-entry into Community territory after being temporarily exported, shall be established;
- (c) the conditions for converting temporary entry into permanent entry shall be determined;
- (d) a Community reference laboratory for one or more of the diseases of equidae listed in Annex I may be designated and the functions, tasks and procedures regarding collaboration with laboratories responsible for diagnosing infectious diseases of equidae in the Member States shall be provided for.

CHAPTER IV

FINAL PROVISIONS

Article 20

Annexes I to IV shall be amended in accordance with the procedure referred to in Article 21(3).

Article 21

1 The Commission shall be assisted by the Standing Committee on the Food Chain and Animal Health set up pursuant to Article 58 of Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety⁽¹⁰⁾.

2 Where reference is made to this paragraph, Articles 5 and 7 of Decision 1999/468/EC shall apply.

The period laid down in Article 5(6) of Decision 1999/468/EC shall be set at three months.

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3 Where reference is made to this paragraph, Articles 5 and 7 of Decision 1999/468/EC shall apply.

The period laid down in Article 5(6) of Decision 1999/468/EC shall be set at 15 days.

Article 22

Directive 90/426/EEC, as amended by the acts listed in Annex V, Part A, is repealed, without prejudice to the obligations of the Member States relating to the time-limits for transposition into national law of the Directives set out in Annex V, Part B.

References to the repealed Directive shall be construed as references to this Directive and shall be read in accordance with the correlation table in Annex VI.

Article 23

This Directive shall enter into force on the 20th day following its publication in the *Official Journal of the European Union*.

Article 24

This Directive is addressed to the Member States.

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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⁽¹¹⁾

Passport No ...

I, the undersigned, certify that⁽¹²⁾ 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...⁽¹³⁾
 — it is not vaccinated against African horse sickness, or,
 it was vaccinated against African horse sickness on ...⁽¹³⁾⁽¹⁴⁾;
- (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,

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- 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⁽¹⁵⁾.

Date	Place	Stamp and signature of the official veterinarian^a

a Name in block capitals and capacity.

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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 <input type="checkbox"/> Assembly centre <input type="checkbox"/> Other <input type="checkbox"/> Name Approval number Address Postal code			I.13. Place of destination Holding <input type="checkbox"/> Assembly centre <input type="checkbox"/> Establishment <input type="checkbox"/> Other <input type="checkbox"/> 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 <input type="checkbox"/> Ship <input type="checkbox"/> Railway wagon <input type="checkbox"/> Road vehicle <input type="checkbox"/> Other <input type="checkbox"/> 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 <input type="checkbox"/> Registered equidae <input type="checkbox"/> Slaughter <input type="checkbox"/> Other <input type="checkbox"/>						
I.26. Transit through third country <input type="checkbox"/> Third country ISO code Exit point Code Entry point BIP unit no.:			I.27. Transit through Member States <input type="checkbox"/> Member State ISO code Member State ISO code Member State ISO code			
I.28. Export <input type="checkbox"/> Third country ISO code Exit point Code			I.29. Estimated journey time			
I.30. Route plan Yes <input type="checkbox"/> No <input type="checkbox"/>						
I.31. Identification of the commodities Species (Scientific name) Identification system						

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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;		
	<i>either</i> ⁽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;		
	<i>or</i> ⁽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 (<i>insert date</i>) and during the 10 days prior to dispatch on (<i>insert date</i>);		
		<i>either</i> ⁽³⁾ [with negative result in each case if it/they was/were not vaccinated against African horse sickness;]		
		<i>or</i> ⁽²⁾ [without increase in antibody count, if it/they was/were vaccinated against African horse sickness;]		
	<i>either</i> ⁽³⁾	II.4. it/they is/are not vaccinated against African horse sickness;		
	<i>or</i> ⁽²⁾	II.4. it/they was/were vaccinated against African horse sickness on (<i>insert date</i>),		
	<i>either</i> ⁽³⁾ [at least two months prior to certification;]			
	<i>or</i> ⁽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:			
<i>either</i> ⁽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,			
	<i>either</i> ⁽²⁾ [six months beginning on the date of the last actual or possible contact with a sick or infected with <i>Trypanosoma equiperdum</i> animal;]			
	<i>or</i> ⁽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 <i>Burkholderia mallei</i> 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;			

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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
	<p>(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;</p> <p>(e) in the case of vesicular stomatitis, six months from the last case;</p> <p>(f) in the case of rabies, one month from the last case;</p> <p>(g) in the case of anthrax, 15 days from the last case;</p> <p>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;]</p> <p>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;</p> <p>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⁾.</p>		
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			

[^{F2}ANNEX IV**AFRICAN HORSE SICKNESS
DIAGNOSIS****Textual Amendments**

- F2** 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⁽¹⁶⁾; 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)⁽¹⁷⁾ 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 MilkTM), 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.

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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 H₂O₂).

Colour development is stopped by adding 50 µl of 3N H₂SO₄ 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 % H₂O₂), 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*

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

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

- 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⁽¹⁸⁾

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.

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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 ($Ct \leq 35$).

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⁽¹⁹⁾*

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⁽²⁰⁾). 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.

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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.

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

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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

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—	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

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- (1) Opinion of 22 April 2009 (not yet published in the Official Journal).
- (2) OJ L 224, 18.8.1990, p. 42.
- (3) See Annex V, Part A.
- (4) OJ L 3, 5.1.2005, p. 1.
- (5) OJ L 224, 18.8.1990, p. 29.
- (6) OJ L 184, 17.7.1999, p. 23.
- (7) OJ L 224, 18.8.1990, p. 55.
- (8) OJ L 157, 10.6.1992, p. 19.
- (9) OJ 121, 29.7.1964, p. 1977.
- (10) OJ L 31, 1.2.2002, p. 1.
- (11) This attestation is not required where there is a bilateral agreement in accordance with Article 6 of Directive 2009/156/EC.
- (12) Valid for 10 days.
- (13) Delete whichever does not apply.
- (14) The vaccination date must be entered in the passport.
- (15) 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.
- (16) [^{F2}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.I., Eds. CRC Press, Boca Raton, Florida, USA, 646-650.]
- (17) [^{F2}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.]
- (18) [^{F2}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.]
- (19) [^{F2}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.]
- (20) [^{F2}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

- F2** 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).