COUNCIL DIRECTIVE
of 26 June 1990
on animal health conditions governing the movement and import from third countries of equidae

90/426/EEC

(OJ No. L 224, 18.8.90, p. 42)

amended by 90/425/EEC (OJ No. L 224 18.08.90 p. 29
amended by 91/496/EEC (OJ No. L 268 24.09.91 p. 56)
amended by 92/130/EEC (OJ No. L 047 22.02.92 p. 26)
amended by 92/36/EEC (OJ No. L 157 10.06.92 p. 28)
amended by ACT of 1994
incorporated by 94/103/EC (OJ L 1, 3.1.1994, p.220)'
amended by (EC) No 806/2003 (OJ No. L 122, 16.05.2003, p. 1)
amended by ACT of 2004

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community, and in particular Article 43 thereof,

Having regard to the proposal from the Commission,

Having regard to the opinion of the European Parliament,

Having regard to the opinion of the Economic and Social Committee,

Whereas

90/426/EEC

Whereas equidae, being live animals, are included in the list of products in Annex II to the Treaty;
Whereas 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 must be laid down at Community level;
Whereas the breeding and rearing of equidae and in particular of horses is generally included in the farming sector; whereas it constitutes a source of income for part of the farming population;
Whereas disparities as regards animal health conditions in the Member States should be eliminated in order to encourage intra-Community trade in equidae;
Whereas, in order to encourage the harmonious development of intra-Community trade, a Community system should be laid down to govern imports from third countries;
Whereas the conditions for the movement on national territory of equidae bearing an identification document should also be regulated;
Whereas, in order to be the subject of trade, equidae must satisfy certain animal health requirements, so as to avoid the spreading of contagious diseases; whereas it appears in particular appropriate to provide for a possible regionalization of restrictive measures;
Whereas transport conditions should be laid down for the same reason;
Whereas, to ensure that those requirements are satisfied provision must be made for the issue by an official veterinarian of a health certificate to accompany the equidae to their place of destination;
Whereas the organization 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 should be fixed within the framework of rules to be laid down for veterinary checks in intra-Community trade in live animals in view of the completion of the internal market;
Whereas provision should be made for the possibility of checks by the Commission; whereas these checks should be carried out in cooperation with the competent national authorities;
Whereas 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;
Whereas the choice of these countries must be based on criteria of a general nature such as the state of health of the livestock, the organization and powers of the veterinary services and the health regulations in force;

Whereas, in addition, imports of equidae should not be authorized from countries infected with contagious or infectious animal diseases which present a risk to Community livestock or which have been free from such infection for too short a period; whereas such considerations are also valid for imports from third countries in which vaccination against such diseases is carried.

Whereas the general conditions applicable to imports from third countries must be supplemented by special conditions drawn up on the basis of the health situation in each of them; whereas the technical nature and the diversity of the criteria on which these 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;

Whereas 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; whereas such rules may include special provisions which may vary according to the third country concerned, and whereas this must be taken into account in drawing up the standard forms of certificates;

Whereas official Community veterinarians should be responsible for verifying that the requirements of this Directive are observed, particularly in third countries;

Whereas the checks carried out upon importation must cover the origin and the state of health of the equidae;

Whereas the Member States must be allowed, on the arrival of equidae in the territory of the Community and during transit to their place of destination, to take all measures, including slaughter and disposal, required for the purpose of safeguarding the health of humans and animals;

Whereas the general rules applicable to the checks to be carried out on importation must be defined within an overall context;

Whereas every Member State must have the right to place an immediate prohibition on imports from a third country when such imports may be dangerous for animal health; whereas in such a case coordination of the attitudes of the Member States with regard to that third country must be assured without delay, without prejudice to possible amendments to the list of countries authorized to export to the Community;

Whereas the provisions of this Directive should be revised in connection with the completion of the internal market;

Whereas provision should be made for a procedure establishing close and effective cooperation between the Commission and the Member States within the Standing Veterinary Committee,

HAS ADOPTED THIS DIRECTIVE:

90/425/EEC

Whereas the Community is to adopt measures designed to establish the internal market progressively over a period expiring on 31 December 1992;

Whereas the harmonious operation of the common organization of the market in animals and products of animal origin implies the dismantling of zootechnical and veterinary barriers to the development of intra-Community trade in the animals and products concerned; whereas, in this respect, the free movement of animals and agricultural products is a fundamental feature of the common organization of markets and should facilitate the rational development of agricultural production and the optimum use of the factors of production;

Whereas, in the veterinary field, frontiers are currently being used for carrying out checks aimed at safeguarding public health and animal health;

Whereas the ultimate aim is to ensure that veterinary checks are carried out at the place of dispatch only; whereas the attainment of this objective implies the harmonization of the basic requirements relating to the safeguarding of animal health;

Whereas, with a view to the completion of the internal market, pending the attainment of this objective, emphasis should be placed on the checks to be carried out at the place of dispatch and in organizing those that could be carried out at the place of destination; whereas such a solution would entail the suspension of veterinary checks at the Community's internal frontiers and whereas, in this context, there is good reason for retaining a health certificate or an identification document, as provided for in Community rules;

Whereas this solution implies increased confidence in the veterinary checks carried out by the State of dispatch, in particular by the setting up of a system for the rapid exchange of information; whereas the dispatching Member State must ensure that such veterinary checks are carried out in an appropriate manner;

Whereas, in the State of destination, spot veterinary checks could be carried out at the place of destination; whereas, however, in the event of a serious presumption of irregularity, the veterinary check could be carried out while the animals and products are in transit and whereas it is possible to continue to provide for the placing into quarantine in areas which have not been harmonized;

Whereas provision must be made for action to be taken where a veterinary check discloses that the consignment is irregular;

Whereas provision should be made for a procedure for resolving conflicts which could arise concerning consignments from a holding, centre or organization;

Whereas provision must be made for protective measures; whereas in this area, especially for reasons of effectiveness, responsibility must rest firstly with the Member State of dispatch; whereas the Commission must be able to act speedily, in particular by way of on-the-spot visits and adopting measures appropriate to the situation;

Whereas in order to be effective, the rules laid down by this Directive should cover all animals and products that are subject, in intra-Community trade, to veterinary requirements;

Whereas, however, in view of the current state of harmonization and pending Community rules, animals and products that are not the subject of harmonized rules should comply with the requirements of the State of destination provided that the latter are in conformity with Article 36 of the Treaty;

Whereas the abovementioned rules should be applied to zootechnical checks;

Whereas the provisions of existing Directives should be adapted to the new rules laid down in this Directive;

Whereas these rules should be re-examined before the end of 1993;
Whereas the Commission should be entrusted with the task of adopting measures for applying this Directive; whereas, to that end, provision should be made for procedures establishing close and effective cooperation between the Commission and the Member States within the Standing Veterinary Committee,

HAS ADOPTED THIS DIRECTIVE:

91/496/EEC

Whereas laying down principles at Community level on the organization of veterinary checks on animals coming from third countries helps to safeguard supplies and ensure market stability while also harmonizing the measures necessary to ensure the protection of animal health;

Whereas Article 23 of 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 (3) provides, in particular, that the Council must lay down the rules and general principles applicable to checks on imports from third countries of animals covered by the said Directive;

Whereas each consignment of animals from third countries must be subjected to documentary and identity checks upon entry into the territory of the Community;

Whereas principles valid throughout the Community should be fixed concerning the organization and follow-up of physical checks to be carried out by the competent veterinary authorities;

Whereas provision must be made for safeguard arrangements; Whereas, in this context, the Commission must be able to act, particularly by visiting the places concerned and adopting measures appropriate to the circumstances;

Whereas, if the checking system is to function smoothly there must be an approval procedure and border inspection posts must be inspected and there should by exchanges of officials empowered to carry out checks on live animals coming from third countries;

Whereas the laying down of common principles at Community level is all the more necessary given that, with the completion of the internal market in prospect, internal border controls are to be abolished;

Whereas Directives 89/662/EEC, 90/425/EEC, and 90/675/EEC should be amended in order to adapt them to this Directive;

Whereas the provision of certain transitional measures of limited duration appears to be necessary in order to facilitate the transition to the new checking arrangements instituted by this Directive;

Whereas the task of adopting measures for the application of this Directive should be entrusted to the Commission,

HAS ADOPTED THIS DIRECTIVE:

92/130/EEC

Whereas in the light of experience gained, some of the wording in the certificates set out in the Annex to Directive 90/426/EEC should be changed so that guarantees relating to certain diseases can be included;

Whereas, in order to avoid confusion, the provisions of Annexes B and C to the said Directive should be reworded;

Whereas the measures provided for in this Decision are in accordance with the opinion of the Standing Veterinary Committee,

HAS ADOPTED THIS DECISION:

92/36/EEC

Where Directive 90/426/EEC lays down animal health conditions governing the movement and import from third countries of equidae; whereas the said Directive indicates the limits of the territory infected with African horse sickness and the rules applicable to Member States not free from the disease;

Whereas Directive 92/35/EEC has laid down the control rules; whereas Directive 90/426/EEC must be amended accordingly in order to take account of these provisions,

HAS ADOPTED THIS DIRECTIVE:

2001/298/EC


(2) On the basis of the conclusions of this report, and in particular in order to draw the attention of the veterinarians in charge of the certification to their responsibilities concerning the protection of animals during transport, it is necessary to supplement the health certificates provided for these animals.


(4) The additional statement introduced in the present Decision does not exempt transporters from their obligations in accordance with Community provisions in force in particular regarding the fitness of animals to be transported.

(5) The measures provided for in this Decision are in accordance with the opinion of the Standing Veterinary Committee,

HAS ADOPTED THIS DECISION:
2002/160/EC


(2) In November 2000 the Community reference laboratory in Algèze, Spain, hosted the annual meeting of the national reference laboratories for African horse sickness of EU Member States. During this meeting scientific evidence was presented that the complement fixation test currently described in Annex D to Directive 90/426/EEC has serious limitations in particular because it is only suitable for detecting antibodies after a recent infection or vaccination. Furthermore, the test is in practice replaced by modern ELISA tests in almost all laboratories in the Community and also in major exporting countries.

(3) The internationally accepted laboratory tests for the detection of antibodies against the African horse sickness virus are described in the Manual of Standards for Diagnostic Tests and Vaccines of the Office International des Epizooties (OIE); however, the current edition mentions only one of the ELISA tests available.

(4) Therefore, it appears appropriate to modify Annex D to Directive 90/426/EEC so as to take into account technical developments and internationally approved standards.

(5) The measures provided for in this Decision are in accordance with the opinion of the Standing Veterinary Committee.

HAS ADOPTED THIS DECISION:

(EC) No. 806/2003


(2) In accordance with the statement of the Council and of the Commission on Decision 1999/468/EC, the provisions relating to committees which assist the Commission in the exercise of its implementing powers, provided for in Decision 87/373/EEC, should be adapted in order to align them with the provisions of Articles 3, 4 and 5 of Decision 1999/468/EC.

(3) The aforesaid statement indicates the methods for adapting the committee procedures, which is automatic provided that this does not affect the nature of the committee provided for in the basic act.

(4) The time limits set in the provisions to be adapted must remain in force. Wherever there is no specific time limit laid down for adopting the implementing measures, the time limit should be set at three months.

(5) The provisions of the instruments providing for recourse to the type I committee procedure established by Decision 87/373/EEC should therefore be replaced by provisions referring to the advisory procedure laid down in Article 3 of Decision 1999/468/EC.

(6) The provisions of the instruments providing for recourse to type IIa and IIb committee procedures established by Decision 87/373/EEC must be replaced by provisions referring to the management procedure provided for in Article 4 of Decision 1999/468/EC.

(7) The provisions of the instruments providing for recourse to type IIIa and IIIb committee procedures established by Decision 87/373/EEC must be replaced by provisions referring to the regulatory procedure provided for in Article 5 of Decision 1999/468/EC.

(8) This Regulation aims purely at aligning committee procedures. The name of the committee relating to these procedures has, where appropriate, been amended.

HAS ADOPTED THIS REGULATION:

2004/68/EC

(1) Council Directive 72/462/EEC of 12 December 1972 on health and veterinary inspection problems upon importation of bovine, ovine and caprine animals and swine, fresh meat or meat products from third countries ensures a high level of animal health protection by laying down the general sanitary requirements for certain imports from third countries.

(2) It is necessary to rationalise and update the animal health provisions concerning international trade in animals provided for in Directive 72/462/EEC due to the evolution of the international standards of the Office International des Epizooties (OIE) and the adoption by this Office of new standards, together with their implications in the framework of the World Trade Organisation (WTO) and its Agreement on the Application of Sanitary and Phytosanitary Measures.

(3) In addition, Council Directive 2002/99/EC of 16 December 2002 laying down the animal health rules governing the production, processing, distribution and introduction of products of animal origin for human consumption replaces the requirements for meat and meat products provided for in Directive 72/462/EEC. It is therefore necessary and appropriate to lay down similar and updated animal health provisions for imports of live ungulate animals into the Community in this Directive.

(4) In order to protect animal health, these new provisions should be extended to cover other ungulate animals that may present a similar risk of disease transmission. However, their application to such animals should be without prejudice to Council Regulation (EC) No 338/97 of 9 December 1996 on the protection of species of wild fauna and flora by regulating trade therein.

(5) Pursuant to Council Directive 90/426/EEC of 26 June 1990 on animal health conditions governing the movement and import from third countries of equidae, imports into the Community of equidae are allowed only from third countries appearing on a list drawn up in accordance with Directive 72/462/EEC. The provisions for establishing lists of third countries for imports of such equidae should be included in Directive 90/426/EEC.

(6) Scientific knowledge concerning the susceptibility and testing of certain animals to diseases changes regularly. A procedure should therefore be established so that the scope of the list of animal species and the diseases to which they are susceptible can be rapidly updated in response to such developments.

(7) In the interests of animal welfare and consistency of Community legislation, the general requirements of Council Directive 91/628/EEC of 19 November 1991 on the protection of animals during transport, in particular as regards watering and feeding, should be taken into account in this Directive.

(8) In the interests of the protection of animal health and consistency of Community legislation, Council Directive 91/496/EEC of 15 July 1991 laying down the principles governing the organisation of veterinary checks on animals entering the Community from third countries should also be taken into account.
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 on the Commission.

The public health and official control rules which apply to meat and meat products by virtue of Directive 72/462/EEC have been replaced by those of Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption, which should apply as from 1 January 2006. The other rules of the said Directive have been replaced by Directive 2002/99/EC, the provisions of which apply as from 1 January 2005, or will be replaced by those of this Directive.

Directive 72/462/EEC should therefore be repealed when all the texts replacing the provisions thereof will be applicable.

It is necessary, however, in the interest of clarity of Community legislation, to repeal certain decisions that are no longer applicable and at the same time to provide for certain implementing rules to remain in force until the necessary measures have been adopted under the new legal framework.

In accordance with the principle of proportionality, it is necessary and appropriate for the achievement of the basic objective of protecting animal health to lay down rules on the conditions for the importation of live ungulate animals. This Directive does not go beyond what is necessary in order to achieve the objectives pursued in accordance with the third paragraph of Article 5 of the Treaty.


Council Directive 92/65/EEC of 13 July 1992 laying down animal health requirements governing trade in and imports into the Community of animals, semen, ova and embryos not subject to animal health requirements laid down in specific Community rules referred to in Annex A(I) to Directive 90/425/EEC, lays down the conditions for the importation into the Community of ungulate animals other than domestic bovine, ovine, caprine, porcine and equine animals, and provides for a list to be laid down of third countries from which Member States may import such animals as well as the health requirements to be met. This Directive should be amended in order to exclude from its scope the animal species covered by the present act.

It is also appropriate to provide that the testing requirements upon importation of live animals covered by Directive 92/65/EEC should be updated or established by committee procedure.

Directives 90/426/EEC and 92/65/EEC should therefore be amended accordingly.

HAS ADOPTED THIS DIRECTIVE:

2006/104/EC

Pursuant to Article 56 of the Act of Accession, where acts of the institutions remain valid beyond 1 January 2007, and require adaptation by reason of accession, and the necessary adaptations have not been provided for in the Act of Accession or its Annexes, the necessary acts are to be adopted by the Council, unless the Commission adopted the original act.

The Final Act of the Conference which drew up the Treaty of Accession indicated that the High Contracting Parties had reached political agreement on a set of adaptations to acts adopted by the institutions required by reason of accession and invited the Council and the Commission to adopt these adaptations before accession, completed and updated where necessary to take account of the evolution of the law of the Union.


HAS ADOPTED THIS DIRECTIVE:

CHAPTER I
General provisions

Article 1

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

Article 2

For the purposes of this Directive:

(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 Directive 90/427/EEC, identified by means of an identification document issued by 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 any international association or organization which manages horses for competition or racing.
(d) 'equidae for slaughter' means equidae intended to be transported either directly or after transit through a market or an approved marshalling centre 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 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 A;
(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 a registered animal originating in a third country and admitted into Community territory for a period of less than 90 days to be fixed by the Commission in accordance with the procedure laid down in Article 24, depending on the health situation in the country of origin.

CHAPTER II
Rules for the movement of equidae

Article 3

Member States shall authorize the movement of equidae registered 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 organized by authorized 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 authorization 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 contagious or infectious disease eradication.

4. The equidae must be identified in the following manner:
   (i) in the case of registered horses, by means of an identification document, as provided for in Directive 90/427/EEC (5), which must certify in particular that Article 5 (5) and (6) have been complied with. The official veterinarian will have to suspend the validity of this document for the period of the prohibitions provided for in paragraph 5 or in Article 5. The document should, 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 by the Commission in accordance with the procedure laid down in Article 24;
   (ii) for equidae for breeding and production, identification by a method to be established by the Commission in accordance with the procedure laid down in Article 24.

Until such time as this method is in use, the officially approved national identification methods shall remain applicable, provided that they are notified to the Commission and the other Member States within three months of the date on which this Directive is adopted.
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:

- 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,
- 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,
- six months in the case of vesicular stomatitis,
- one month from the last recorded case, in the case of rabies,
- 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 measures for hippodromes and racecourses, and shall notify the Commission of the nature of any derogations granted.

6. Where 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 of notification of this Directive outlining in particular:

- the distribution of the disease on its territory,
- the reasons for the programme, taking into consideration the significance of the disease and its cost/benefit advantages,
- the geographical area in which the programme will be implemented,
- the status categories to be applied to establishments, the standards which must be attained for each species and the test procedures to be used,
- the programme monitoring procedures,
- the action to be taken if, for any reason, a holding loses its status,
- the measures to be taken if the results of the tests carried out in accordance with the provisions of the programme are positive,
- 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 laid down in Article 24. 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 laid down in Article 25. 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.

**Article 5**

1. A Member State which is not free of African horse sickness within the meaning of Article 2 (f) 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 3 of this Article.

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

- clinical, serological (in unvaccinated animals) and/or epidemiological evidence has revealed the presence of African horse sickness in the past two years, or
- vaccination against African horse sickness has been carried out in the past 12 months.
(b) The part of the territory considered to be infected with African horse sickness must comprise a minimum:
- a protection zone with a radius of at least 100 km around any centre of infection,
- a surveillance zone at least 50 km extending beyond the protection zone, in which no vaccination has been carried out in the last 12 months.

(c) The rules controlling the combat measures relating to the territories and zones referred to in points (a) and (b) and the relevant derogations are specified in Directive 92/35/EEC (*).

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

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

3. A Member State may dispatch from the territory referred to in paragraph 2 (b) 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 laid down in Article 25;
(b) they must show no clinical symptom of African horse sickness on the day of the inspection referred to in Article 4 (1);
(c) if they have not been vaccinated against African horse sickness, they must have undergone and reacted negatively to a complement fixation test for African horse sickness as described in Annex D, 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;
- if they have been vaccinated, they must not have undergone vaccination during the previous two months and must have undergone the fixation test described in Annex D at the aforementioned intervals without having recorded an increase in the antibody count. Under the procedure laid down in Article 24, the Commission may, following the opinion of the Scientific Veterinary Committee, recognize other monitoring methods;
(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 and registered equidae, in particular by means of the identification document, may grant one another derogations from the provisions of the second sentence of Article 4 (1) and the second indent of Article 8 (1) on a reciprocal basis.

They shall notify the Commission thereof.

Article 7

1. The equidae must be transported, as soon as possible, from the holding of origin either directly or via an approved market or marshalling centre as defined in Article 3 (6) of Directive 64/432/EEC 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. 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 mentions such derogation.

In the case of granting such a derogation 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:
   - registered equidae which leave their holdings are accompanied by the identification document laid down in Article 4 (4) together - if they are intended for intra-Community trade - with the attestation provided for in Annex B,
   - equidae for breeding, production and slaughter are, during their transportation, accompanied by a health certificate complying with Annex C to this Directive.

The certificate, or in the case of an identification document, the form containing the health particulars, 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 certificate is 10 days. The certificate must consist of a single sheet.

2. Imports of equidae other than registered equidae may be covered by a single health certificate per consignment rather than by the individual certificate referred to in the second indent of paragraph 1.

Article 9

The rules laid down in Council Directive 90/425/EEC of 26 June 1990 concerning veterinary and zootechnical checks in intra-Community trade in certain live animals and products with a view to the completion of the internal market (*), shall apply in particular to checks at origin, to the organization 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.

(*) OJ No L 224, 18. 8. 1990, p. 29.

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 laid down in Article 24.

CHAPTER III

Rules for imports from third countries

Article 11

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

2. Until the date of entry into force of the decisions adopted pursuant to Articles 12 to 16, the Member States shall apply to imports of equidae from third countries conditions at least equivalent to those resulting from the application of Chapter II.

Article 12

1. The importation of equidae into the Community shall only be authorised from third countries that appear on a list or lists to be drawn up or amended in accordance with the procedure referred to in Article 24(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 24(2) that the authorisation provided for in the preceding sub-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 lists provided for in paragraph 1 are 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 Office International des Epizooties (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 A 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 A 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;
   (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 all lists drawn up or amended as provided for in paragraph 1 to be made available to the public. Those lists 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 24(2).

5. Detailed rules for the application of this Article and criteria for including third countries or parts of third countries in the lists provided for in paragraph 1 may be adopted in accordance with the procedure referred to in Article 24(2).

### Article 13

1. The equidae must come from third countries:
   (a) free from African horse sickness;
   (b) which have been free for two years from Venezuelan equine encephalomyelitis (VEE);
   (c) which have been free for six months from dourine and glanders.

2. The Commission may, in accordance with the procedure laid down in Article 24:
   (a) decide that the provisions of paragraph 1 shall apply to only a 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 (3) must be complied with;
   (b) 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 regionalization, 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 authorized 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 and the categories of equidae, in accordance with the procedure laid down in Article 24 for imports of equidae from that country.

The reference basis for fixing animal health conditions in accordance with paragraph 1 shall be the standards laid down in Articles 4 and 5; and

(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 (ii) - have reacted negatively to a serological test or to a virus isolation test or to any other test recognized in accordance with the procedure laid down in Article 24 which would guarantee freedom from the virus.

In accordance with the procedure laid down in Article 24, and following the opinion of the Scientific Veterinary Committee, the Commission may define the categories of male equidae to which this requirement shall apply.

**Article 16**

1. The equidae must be identified in accordance with Article 4 (4) and accompanied by a certificate drawn up by an official veterinarian of the exporting third country. This 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 States 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 certificate must be drawn up on a form complying with a model established in accordance with the procedure laid down in Article 24.

**Article 17**

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.

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 according to the procedure provided for in Article 25.

The experts from the Member States who are to be entrusted with these checks shall be appointed by the Commission, acting on a proposal from the Member States.
These 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 these checks shall be determined in accordance with the procedure laid down in Article 24.

**Article 18**

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 a market or a marshalling centre, and, in accordance with animal health requirements, be slaughtered within a period of time 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 laid down in Article 24, 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 19**

The Commission, acting in accordance with the procedure laid down in Article 24

(i) may decide that imports from a third country or part of a third country are to be confined to particular species or categories;

(ii) shall, notwithstanding Article 15, establish 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;

(iii) shall determine the conditions for converting temporary entry into permanent entry.

(iv) may designate a Community reference laboratory for one or more of the diseases of equidae listed in Annex A and shall stipulate the functions, tasks and procedures regarding collaboration with laboratories responsible for diagnosing infectious diseases of equidae in the Member States.

**Article 20**

deleted

**Article 21**

deleted

**CHAPTER IV**

**Final provisions**

**Article 22**

The provisions of this Directive, and in particular those contained in the second sentence of Article 4 (1) and in Articles 6, 8 and 21, shall be re-examined before 1 January 1993 in the framework of the proposals relating to the completion of the internal market, on which the Council will decide by a qualified majority.

**Article 23**

The Annexes to this Directive shall be amended by the Commission in accordance with the procedure provided for in Article 25.

**Article 24**

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 (*).

2. Where reference is made to this Article, 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.
3. The Committee shall adopt its Rules of Procedure.

**Article 25**

1. The Commission shall be assisted by the Standing Committee on the Food Chain and Animal Health.

2. Where reference is made to this Article, 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 26**

Article 34 of Directive 72/462/EEC shall apply to the requirements set out in Chapter III of this Directive.

**Article 27**

Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive no later than 1 January 1992. They shall forthwith inform the Commission thereof.

**Article 28**

This Directive is addressed to the Member States.

Done at Luxembourg, 26 June 1990.

For the Council

The President

M. O'KENNEDY
ANNEX A

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 B

HEALTH INFORMATION (*)

Passport No....................

I, the undersigned, certify (*) that the equine animal described 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/third country which is the subject of restrictions for reasons of African horse sickness (c),
    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 (3) of Directive 90/426/EEC in the quarantine station of.................................................. between................... and.................................................(c);
    - it is not vaccinated against African horse sickness, or it was vaccinated against African horse sickness on........................... (c) (d);

(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 disease 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 inspection it was fit to be transported on the intended journey in accordance with the provisions of Directive 91/628/EEC (e).

Date Place Stamp and signature of the official veterinarian (1)

(1) Name in block capitals and capacity.
(*) This information is not required where there is a bilateral agreement in accordance with Article 6 of Directive 90/426/EEC.
(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.
ANNEX C

MODEL
HEALTH CERTIFICATE
for trade between Member States of the EEC

EQUIDAE

No: ........................................................................................................................................................................................

Member State of dispatch: ..................................................................................................................................................................

Ministry responsible: ....................................................................................................................................................................

Territorial Department responsible: ..............................................................................................................................................

I. Number of equidae:

II. Identification of equidae:

<table>
<thead>
<tr>
<th>Number of equidae (1)</th>
<th>Species horse, ass, mule, hinny</th>
<th>Breed Age Sex</th>
<th>Method of identification and identification (2)</th>
</tr>
</thead>
<tbody>
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</table>

(1) In the case of animals for slaughter, nature of the special mark.
(2) A passport identifying the equine animal may be attached to this certificate provided that its number is stated.

III. Origin and destination of animal/s:

The animal/s is/are to be sent
from: .............................................................................................................................................................................

(Place of export)

to: .............................................................................................................................................................................

(Member State and place of destination)

Name and address of consignor: ............................................................................................................................................

Name and address of consignee: ............................................................................................................................................

IV. Health information (3)

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

1. it/they has/have been examined today and show/s no clinical sign of disease;
2. it/they is/are not intended for slaughter under a national programme of contagious or infectious disease eradication;
3. it/they does/do not come from the territory or part of the territory of a Member State/third country which is the subject of restrictions for reasons of African horse sickness,
or
- it/they come/s from the territory or part of the territory of a Member State which was the subject of restrictions for reasons of African horse sickness and has/have undergone, with satisfactory results, the tests provided for in Article 5 (3) of Directive 90/426/EEC in the quarantine station of between and (3);
- it/they is/are not vaccinated against African horse sickness,
or
- it/they was/were vaccinated against African horse sickness on (4);

4. it/they has/have 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 disease located on the holding have been slaughtered and the premises desinfected during 30 days, beginning on the day on which the animals were destroyed and the premises desinfected, except in the case of anthrax, where the period of prohibition is 15 days;

5. 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.

6. at the time of inspection it/they was/were fit to be transported on the intended journey in accordance with the provisions of Directive 91/628/EEC (d).

V. This certificate is valid for 10 days.

Done at .........................................................., date.........................................................

..........................................................

(signature of the official veterinarian) (f)

..........................................................

(Signature)

(Name in capital letters and capacity of signing veterinarian) (f)

(a) This information is not required where there is a bilateral agreement in accordance with Article 6 of Directive 90/426/EEC.
(b) Delete whichever does not apply.
(c) In Germany 'Beamter Tierarzt'; in Belgium 'Inspecteur vétérinaire' or 'Inspecteur Dierenarts'; in France 'Vétérinaire officiel'; in Italy 'Veterinario ufficiale'; in Luxembourg 'Inspecteur vétérinaire'; in the Netherlands 'Officicel Dierenarts'; in Denmark 'Embeds Dyrlæge'; in Ireland 'Veterinary Inspector'; in the United Kingdom 'Veterinary Inspector'; in Greece 'Episimos ktiniatros'; in Spain 'Inspector Veterinario'; and in Portugal 'Inspector Veterinário'.
(d) 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.
ANNEX D
AFRICAN HORSE SICKNESS
DIAGNOSIS

Reagents for the enzyme-linked immunoassorbent assays (ELISA) described below may be obtained from the European Community Reference Laboratory or the OIE Reference Laboratories for African horse sickness.

1. COMPETITIVE ELISA FOR THE DETECTION OF ANTIBODIES TO AFRICAN HORSE SICKNESS VIRUS (AHSV) (PRESCRIBED TEST)

Competitive ELISA is used to detect specific AHSV antibodies in sera from any species of equidae. The broad spectrum, polyclonal, immune anti-AHSV guinea-pig serum (hereinafter “guinea-pig antiserum”) is serogroup specific and is able to detect all known serotypes of AHS virus.

The principle of the test is the interruption of the reaction between AHSV antigen and a guinea-pig antiserum by a test serum sample. AHSV antibodies in the test serum sample will compete with those in the guinea-pig antiserum resulting in a reduction in the expected colour (following the addition of enzyme labelled anti-guinea-pig antibody and substrate). Sera can be tested at a single dilution of 1 in 5 (spot test method) or may be titrated (serum titration method) to give dilution end-points. Inhibition values higher than 50 % may be regarded as positive.

The test protocol described hereinafter is used in the Regional Reference Laboratory for African horse sickness in Pirbright, United Kingdom.

1.1. Test procedure

1.1.1. Preparation of plates

1.1.1.1. Coat ELISA plates with AHSV antigen extracted from infected cell cultures and diluted in carbonate/bicarbonate buffer, pH 9.6. Incubate the ELISA plates overnight at 4 °C.

1.1.1.2. Wash plates three times by flooding and emptying the wells with phosphate buffered saline (PBS), pH 7.2 to 7.4, and blot dry on absorbent paper.

1.1.2. Control wells

1.1.2.1. Titrate the positive control sera in a twofold dilution series, from 1 in 5 to 1 in 640, across column 1 in blocking buffer (PBS containing 0.05 % (v/v) Tween-20, 5.0 % (w/v) skimmed-milk powder (Cadbury's Marvel™) and 1 % (v/v) adult bovine serum) to give a final volume of 50 µl/well.

1.1.2.2. Add 50 µl of the negative control serum at a dilution of 1 in 5 (10 µl serum + 40 µl blocking buffer) to wells A and B of column 2.

1.1.2.3. Add 100 µl/well of blocking buffer to wells C and D of column 2 (blank).

1.1.2.4. Add 50 µl of blocking buffer to wells E, F, G and H of column 2 (guinea pig control).

1.1.3. Spot test method

1.1.3.1. Add a 1 in 5 dilution of each test serum in blocking buffer to duplicate wells of columns 3 to 12 (10 µl sera + 40 µl blocking buffer).

or

1.1.4. Serum titration method

1.1.4.1. Prepare a twofold dilution series of each test sample (1 in 5 to 1 in 640) in blocking buffer across eight wells of single columns (3 to 12).

then

1.1.5. Add 50 µl of guinea pig antiserum, pre-diluted in blocking buffer, to all wells except the blank wells of the ELISA plate (all wells now contain a final volume of 100 µl).

1.1.5.1. Incubate for 1 hour at 37 °C on an orbital shaker.

1.1.5.2. Wash plates three times and blot dry as before.

1.1.5.3. Add 50 µl of rabbit anti-guinea-pig horseradish peroxidase (HRP) conjugate pre-diluted in blocking buffer to each well.

1.1.5.4. Incubate for 1 hour at 37 °C on an orbital shaker.

1.1.5.5. Wash plates three times and blot dry as before.

1.1.6. Chromogen

Prepare the chromogen OPD (OPD = ortho-phenyldiamine) solution according to the manufacturer's instructions (0.4 mg/ml in sterile distilled water) just before use. Add substrate (hydrogen peroxide = H₂O₂) to give a final concentration of 0.05 % (v/v) (1 in 2000 of a 30 % solution of H₂O₂). Add 50 µl of the OPD solution to each well and leave plates on the bench for 10 minutes at ambient temperature. Stop the reaction by the addition of 50 µl/well of 1M sulphuric acid (H₂SO₄).

1.1.7. Reading

Read spectrophotometrically at 492 nm.

1.2. Expression of results
1.2.1. Using a software package print out the optical density (OD) values, and the percentage inhibition (PI) for test and control sera based on the mean value recorded in the four guinea pig control wells. The data expressed as OD and PI values are used to determine whether the test has performed within acceptable limits. The upper control limits (UCL) and lower control limits (LCL) for the guinea pig control are between OD values 1.4 and 0.4, respectively. The end-point titre for the positive control based on 50 % PI should be 1 in 240 (within a range from 1 in 120 to 1 in 480). Any plate that fails to conform to the above criteria must be rejected. However, if the positive control serum titre is greater than 1 in 480 and the test samples are still negative then the negative test samples can be accepted.

The duplicate negative control serum wells and the duplicate blank wells should record PI values between +25 % and −25 %, and between +95 % and +105 %, respectively. Failure to be within these limits does not invalidate the plate but does suggest that background colour is developing.

1.2.2. The diagnostic threshold (cut-off value) for test sera is 50 % (PI 50 %). Samples recording PI values greater than 50 % are recorded as positive. Samples recording PI values lower than 50 % are recorded as negative.

Samples that record PI values above and below the threshold for the duplicate wells are considered doubtful. Such samples may be re-tested in the spot test and by titration. Positive samples may also be titrated to provide an indication of the degree of positivity.

<table>
<thead>
<tr>
<th>Spot test layout</th>
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<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>+ve cont.</td>
</tr>
<tr>
<td>Test sera</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
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</tr>
<tr>
<td>F</td>
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<tr>
<td>G</td>
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<tr>
<td>H</td>
</tr>
</tbody>
</table>

−ve cont = negative control.
+ve cont = positive control.
GP cont = guinea pig control.

<table>
<thead>
<tr>
<th>Test sera</th>
</tr>
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<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>+ve cont.</td>
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<tr>
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<td>H</td>
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</table>

−ve cont = negative control.
2. INDIRECT ELISA FOR THE DETECTION OF ANTIBODIES TO AFRICAN HORSE SICKNESS VIRUS (AHSV) (PREScribed TEST)

The test described hereinafter is in accordance with the test description in Chapter 2.1.11 of the OIE Manual of Standards for Diagnostic Tests and Vaccines, fourth edition, 2000.

The recombinant VP7 protein has been used as antigen for AHS virus antibody determination with a high index of sensitivity and specificity. Other advantages are that it is stable and not infective.

2.1. Test procedure

2.1.1. Solid phase

2.1.1.1. ELISA plates are coated with recombinant AHSV-4 VP7 diluted in carbonate/bicarbonate buffer, pH 9.6. Incubate plates overnight at 4 °C.

2.1.1.2. 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.

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

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

2.1.2. Test samples

2.1.2.1. 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.

2.1.2.2. Wash the plates as described in step 2.1.1.2.

2.1.3. Conjugate

2.1.3.1. Dispense 100 µl/well of horseradish-peroxidase (HRP)-conjugated anti-horse gamma-globulin diluted in PBS + 5 % milk + 0.05 % Tween 20, 20, 100 µl per well. Incubate for 1 hour at 37 °C.

2.1.3.2. Wash the plates as described in step 2.1.1.2.

2.1.4. Chromogen/Substrate

2.1.4.1. Add 200 µl/well of chromogen/substrate solution (10 ml of 80.6 mM DMAB (dimethyl amino-benzaldehyde)+ 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.

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

2.2. Interpretation of the results

2.2.1. Calculate the cut-off value by adding 0.6 to the value of the negative control (0.6 is the standard deviation derived with a group of 30 negative sera).

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

2.2.3. Test samples giving absorbance values greater than the cut-off + 0.15 are regarded as positive.

2.2.4. Test samples giving intermediate absorbance values are doubtful and a second technique must be employed to confirm the result.

3. BLOCKING ELISA FOR THE DETECTION OF ANTIBODIES TO AFRICAN HORSE SICKNESS VIRUS (AHSV) (PREScribed TEST)

The blocking ELISA is designed to detect specific AHSV antibodies in sera from any susceptible species. VP7 is the major, antigenic, viral protein of AHSV, and is conserved within the nine serotypes. Because the monoclonal antibody (Mab) is also directed against the VP7, the assay will give a high level of sensitivity and specificity. Further, the recombinant VP7 antigen is completely innocuous and therefore guarantees a high degree of safety.

The principal of the test is the interruption of the reaction between the recombinant VP7, as the antigen bound to the ELISA plate and the conjugated Mab specific for the VP7. Antibody in the test sera will block the reaction between the antigen and the Mab resulting in a reduction in colour.

The test described hereinafter is carried out in the European Community Reference Laboratory for African horse sickness in Algete, Spain.

3.1. Test procedure

3.1.1. ELISA plates

3.1.1.1. Coat ELISA plates with recombinant AHSV-4 VP7 diluted in carbonate/bicarbonate buffer, pH 9.6. Incubate overnight at 4 °C.
3.1.1.2. Wash the plates five times with phosphate buffered saline (PBS) containing 0.05 % (v/v) Tween 20 (PBST).

3.1.1.3. Stabilise the plate by treatment with a stabilising solution (in order to allow long term storage at 4 °C without loss of activity) and blot dry onto adsorbent material.

3.1.2. Test samples and controls

3.1.2.1. For screening: dilute test sera and controls 1 in 10 directly on the plate in PBST to give a final volume 100 µl/well. Incubate for 1 hour at 37 °C.

3.1.2.2. For titration: prepare a twofold dilution series of test sera and positive controls (100 µl/well) from 1 in 10 to 1 in 1 280 across eight wells. Negative control is tested at 1 in 10 dilution.

3.1.3. Conjugate

Add 50 µl/well of pre-diluted horseradish-peroxidase (HRP)-conjugated Mab (monoclonal antibodies specific for VP7) to each well and mix gently to ensure homogeneity. Incubate for 30 minutes at 37 °C.

3.1.4. Wash the plates five times with PBST and blot dry as above.

3.1.5. Chromogen/Substrate

Add 100 µl/well of chromogen/substrate solution (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).

3.1.6. Reading

Read at 405 nm in an ELISA reader.

3.2. Interpretation of the results

3.2.1. Assay validation

The test is valid when the optical density (OD) of negative control (NC) is higher than 1.0 and the OD of positive control (PC) is lower than 0.2.

3.2.2. Cut-off calculation

Positive cut-off = NC – ((NC – PC) x 0.3)
Negative cut-off = NC – ((NC – PC) x 0.2)

Where, NC is the OD of the negative control and PC the OD of positive control.

3.2.3. Interpretation of results

Samples with OD lower than positive cut-off should be considered as positives to AHSV antibodies.

Samples with OD higher than negative cut-off should be considered negatives for AHSV antibodies.

Samples with OD between these two values should be considered doubtful and the animals resampled after two to three weeks.