II

(Acts whose publication is not obligatory)

COMMISSION

COMMISSION DECISION

of 27 September 1983

concerning animal health conditions and veterinary certification for the importation of domestic animals of the bovine and porcine species from Canada

(83/494/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 72/462/EEC of 12 December 1972 on health and veterinary inspection problems upon importation of bovine animals and swine and fresh meat from third countries (1), as last amended by Directive 83/91/EEC (2), and in particular Article 8 thereof,

Whereas, following a Community veterinary mission, it appears that the animal health situation in Canada is controlled by well-structured and organized veterinary services which can offer satisfactory guarantees concerning diseases which might be transmitted through importation of domestic animals of the bovine and porcine species ;

Whereas, in addition, the responsible veterinary authorities of Canada have confirmed that Canada has during the past 12 months been free from rinderpest, foot-and-mouth disease, contagious bovine pleuropneumonia, blue tongue, African swine fever, classical swine fever, contagious porcine paralysis (Teschen disease), swine vesicular disease and vesicular exanthema, that it has during the past six months been free from contagious vesicular stomatitis and that no vaccinations have been carried out against those diseases during those periods ;

Whereas the responsible veterinary authorities of Canada have undertaken to notify the Commission of the European Communities and the Member States, by telex or telegram, within 24 hours, of the confirmation of the occurrence of any of the abovementioned diseases or the adoption of vaccination against them, or within the appropriate period of proposed changes in the Canadian tuberculosis eradication programme, the Canadian brucellosis eradication programme or Canadian import rules concerning bovine animals and swine and semen and embryos thereof ;

Whereas the responsible veterinary authorities of Canada have undertaken to officially supervise the issue of certificates arising from the Commission Decision on imports of bovine animals and swine into the European Economic Community from Canada and ensure that all relevant back-up certificates and statements remain on official file for at least 12 months following dispatch of the animals ;

Whereas animal health conditions and veterinary certification must be adopted according to the animal health situation of the non-member country concerned ; whereas, in consideration of the epizootiological character of blue tongue, additional precautions may be taken by Member States ;

(1) OJ No L 302, 31. 12. 1972, p. 28.
(2) OJ No L 59, 5. 3. 1983, p. 34.

Whereas where quarantine is required, it is necessary to ensure by means of an authorization document that the necessary arrangements have been made in order that it may be carried out in an orderly way, taking into account in particular the capacities of the designated quarantine stations ; whereas this document is to be issued if these conditions are met, and it may also require that during quarantine and transport the animals come into contact only with animals of similar health status ;

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

HAS ADOPTED THIS DECISION :

Article 1

1. Member States shall authorize the importation of animals from Canada as follows :

(a) domestic animals of the bovine species for breeding or production conforming to the guarantees laid down in the health certificate in accordance with Annex A, which must accompany the consignment together with an authorization document. However, such authorization may be restricted to certain periods of the year and/or restricted to non-pregnant animals ;

(b) domestic animals of the porcine species for breeding conforming to the guarantees laid down in the health certificate in accordance with Annex B, which must accompany the consignment together with an authorization document ;

(c) the authorization documents referred to in paragraphs (a) and (b) above may request that animals during quarantine and transportation shall come into contact only with animals of similar health status.

2. Member States shall not authorize the import of domestic animals of the bovine or porcine species other than as indicated in paragraph 1.

3. (a) Should the tests carried out in the pre-embarcation quarantine premises referred to in Annex A (V) (10) and Annex B (V) (10) produce other than negative results, any positive animals shall be removed. Before authorizing the importation of one or more of the remaining animals from that quarantine premises, the Member State of destination together with the veterinary authorities of Canada shalll ensure that such animals continue to conform to the health guarantees referred to in paragraph 1.

(b) Where more than one Member State is involved in the simultaneous use of the quarantine premises the action referred to at (a) above shall be coordinated by the Member States concerned in conjunction with the veterinary authorities of Canada.

(c) The Member States shall furnish a report to the Commission on serious incidents which may occur in quarantine together with details of the action taken.

Article 2

1. Following the health inspection (import control) carried out pursuant to Article 12 of Directive 72/462/EEC at a frontier post in a Member State and the endorsement of the certificate accompanying each consignment of bovine animals or swine indicating that the animals have been admitted, they may be placed in isolation at a place and for a period to be designated by the Member State of destination and whilst there may be subjected to treatment as considered necessary by the Member State or to one or more of the tests described in Annex C according to the species concerned. If results are other than negative, additional more specific tests may be applied.

2. The Member State of destination shall take all necessary measures to prevent contamination of indigenous herds.

Article 3

1. By way of derogation from Article 1 (1) (a), in the case of bulls for direct entry to a semen collection centre, Member States may not apply the provisions of Annex A (V) (5) (iii), second ident. In this case the bulls shall, after importation, be placed in isolation for a period of at least nine months. During this period they shall be subjected to two agar gel immuno diffusion tests for enzootic bovine leukosis according to the provisions of Annex C, with an interval of four months between tests, the second to be completed at the end of the isolation period when the animals are at least 18 months of age.

2. (a) By way of derogation from Article 1 (1) (a), in the case of bulls and non-pregnant females, Member States are empowered not to apply the provisions of Annex A (V) (5) (iii), second indent. In this case the animals shall be at least 18 months of age at the time of loading provided for in subparagraph 13 of Annex A and in the case of females shall be non-pregnant immediately prior to entry into isolation as provided for in subparagraph 6 of Annex A and at the time of loading.

(b) Member States making use of this derogation shall isolate the animals on importation and subject them to an agar gel immuno diffusion test for enzootic bovine leukosis according to the provisions of Annex C two months after importation and again four months later. Any animals showing a positive result to this test shall be slaughtered or re-exported.

(c) Member States shall ensure that animals imported by way of this derogaiton shall remain traceable for two years following importation during which time they shall be subjected to further agar gel immuno diffusion tests for enzootic bovine leukosis according to the provisions of Annex C.

(d) Member States shall, by 31 December 1985 at the latest, submit a report to the Commission on the animals imported under this derogation.

Article 4

This Decision shall apply from 1 July 1984 at the latest.

Article 5

This Decision is addressed to the Member States.

Done at Brussels, 27 September 1983.

For the Commission

Poul DALSAGER

Member of the Commission

ANNEX A

HEALTH CERTIFICATE (1)
for domestic animals of the bovine species for breeding or production intended for consignment to the European Economic Community

No .....

Exporting country : CANADA

Competent ministry : CANADIAN DEPARTMENT OF AGRICULTURE

Competent issuing authority : CANADIAN DEPARTMENT OF AGRICULTURE

I. **Number of animals :** .....
(In words)

II. **Identification of animals**

Number of animals	Cow, bull, ox heifer or calf	Breed	Age	Official marks, other marks or brands (State No and position)

III. **Origin of animals**

The animals have originated from the following herd/herds (2) :

Official mark or brand	Herd of origin (Address and province)

IV. **Destination of animals**

The animals will be sent

from .....
(Place of loading)

to .....
(Member State and place of destination)

entering the territory of the Community

at .....
(Community frontier post)

by : Aircraft (Flight No .....)

Ship (Name .....) (3)

on .....
(Expected date and time of arrival)

Name and address of consignor : .....

.....

Name and address of consignee : .....

.....

V. Attestation of health

I, the undersigned, being an official veterinarian designated by the competent central authority of Canada, certify that after due enquiry and to the best of my knowledge and belief the animals described above meet the following requirements :

1. They,

   (i) were conceived, born and reared in and have not at any time been outside Canada,

   (ii) are not the progeny of animals which are known to have failed to pass the agar gel immuno-diffusion test for blue tongue,

   (iii) originate from herds which have been in existence for at least three years ;

2. They have been identified by an officially approved mark or brand as indicated in paragraph II above ;

3. They are not animals which are to be destroyed under a contagious or infectious-disease eradication programme ;

4. (i) They have not been vaccinated against brucellosis, Johne's disease, or anaplasmosis,

   (ii) They have/have not been vaccinated against bovine virus diarrhoea [3] [5],

   (iii) They have/have not been vaccinated against infectious bovine rhino-tracheitis/infectious pustular vulvo-vaginitis [3] [5] ;

5. They originate from herds :

   (i) which are recognized by the central veterinary authorities of Canada as being tuberculosis-free herds under the Canadian tuberculosis eradication programme,

   (ii) which are recognized by the central veterinary authorities of Canada as being brucellosis-free herds in accordance with the Canadian brucellosis eradication programme 1980 and in which no animal has been vaccinated against brucellosis in the preceding three years [2] and in which all bovine animals have been free of clinical signs of brucellosis for at least six months immediately prior to the isolation period provided for in subparagraph 6,

   (iii) in which :

   — no facts have been brought to the attention of the undersigned or other official of the government of Canada which lead to the conclusion that a case of enzootic bovine leukosis has occurred in the herd within the previous three years [2], the owner having declared in writing that he has no knowledge of such facts and, in addition, that the animals intended for export to the European Economic Community have either been born and reared in the said herd or have remained an integral part of it during the previous 12 months [2],

   — during the previous 12 months [2] all the bovine animals over 24 months of age on the date of the test and forming part of the herd from which they come have reacted negatively to the agar gel immuno-diffusion test for enzootic bovine leukosis [3] [5] [6],

   (iv) in which, immediately prior to the isolation period provided for in subparagraph 6, no evidence has been recorded which leads to a conclusion that :

   — anthrax has occurred during the previous 30 days,

   — infectious bovine rhino-tracheitis/infectious pustular vulvo-vaginitis and bovine virus diarrhoea have occurred during the previous three months,

   — rabies, campylobacteriosis (C. fetus), trichomoniasis (T. foetus), Q fever and leptospirosis have occurred during the previous six months,

   — pathogenic mycoplasmosis and anaplasmosis have occurred during the previous 12 months, or,

   — Johne's disease has occurred during the previous two years ;

6. They have, following a clinical examination at which they were found to be free from symptoms of communicable disease, been isolated from other animals of their herd of origin for a period of not less than 21 days. In the case of bovine animals to which Article 3 of Decision 83/494/EEC applies this period has been not less than two months [5].

7. They have, at the beginning of the isolation period referred to above been subjected to the following tests according to the provisions of Annex C of Commission Decision 83/494/EEC with negative results, the tests where appropriate having been performed at an officially approved laboratory :

   (i) the single intradermal tuberculin test,

   (ii) the serum agglutination test and the complement fixation test for bovine brucellosis,

   (iii) the agar gel immuno-diffusion test for enzootic bovine leukosis,

   (iv) the agar gel immuno-diffusion test for blue tongue and epizootic haemorrhagic disease,

   (v) either the micro-agglutination test for leptospirosis (types L. pomona, grippotyphosa, hardjo, and sejroe) ; and/or they have, during the quarantine period referred to in subparagraph 10 and after completion of the tests referred to therein with negative results (with the exception of tests for leptospirosis), received an injection of dihydrostreptomycin (25 mg per kg of live body weight) (3) (5),

   (vi) the serum neutralization test for infectious bovine rhino-tracheitis/infectious pustular vulvo-vaginitis in the case of animals not vaccinated against that disease (5),

   (vii) the serum neutralization test for bovine virus diarrhoea in the case of animals not vaccinated against that disease (5),

   (viii) in the case of cows in milk a clinical examination for mastitis and an analysis of their milk to determine an inflammatory condition or identify a specific pathogenic micro-organism ;

8. They have, following the completion of the tests laid down in subparagraph 7 above and of the 21-day isolation period (two months in the case of animals to which Article 3 (2) of Decision 83/494/EEC applies) (3) (5), been examined and found to be free from symptoms of communicable disease and evidence of ectoparasites or warbles ;

9. That, within 24 hours following the examination referred to in subparagraph 8 above they have been directly transported to the pre-embarkation quarantine premises referred to at subparagraph 10 below without coming into contact with cloven-hoofed animals other than bovine animals or swine meeting the provisions of Commission Decision 83/494/EEC, in vehicles which have been cleansed and disinfected under the supervision of an official of the Canadian veterinary services ;

10. That they have been resident in a pre-embarkation quarantine premises officially approved and supervised by the Canadian veterinary services until the tests (4) referred to in subparagraph 7 have been repeated with negative results. Tests for leptospirosis have/have not been repeated (3) (5). These tests have not been commenced until the animals have been resident in the quarantine premises for at least 21 days and, where appropriate, have been performed at an officially approved laboratory. In the case of bovine animals to which Article 3 of Decision 83/494/EEC applies this period has been 30 days (5). The tuberculin test was commenced on ..... (date of injection) ;

11. That following the expiry of the quarantine period and the completion of the tests referred to in subparagraph 10 above, they were, following a clinical examination, at which they were found to be free of symptoms of communicable diseases, transported directly to the place of loading for consignment to the European Community, without coming into contact with cloven-hoofed animals other than bovine animals or swine meeting the provisions of Commission Decision 83/494/EEC, in vehicles which have been cleansed and disinfected under the supervision of an official of the Canadian veterinary services ;

12. That in the case of animals to which Article 3 (2) of Decision 83/494/EEC applies the female animals were non-pregnant immediately prior to entry into isolation provided for in subparagraph 6 and that at the time of loading all animals to which this derogation applies are at least 18 months of age and in the case of females are non-pregnant (3) (5) ;

13. That at the place of loading they were placed on board a ship or aircraft in accommodation which has been cleansed and disinfected under the supervision of an official of the Canadian veterinary services and that no cloven-hoofed animals other than bovine animals or swine meeting the provisions of Commission Decision 83/494/EEC have been loaded onto the said ship or aircraft ;

VI. **This certificate is valid for 21 days from the date of loading.**

Done at ....., on .....
(Date of loading)

Seal

.....
(Signature of official veterinarian)

.....
(Name in capital letters and title)

VII. **Declaration by captain of the aircraft or master of the ship**

I, the undersigned, captain of aircraft (flight No .....)/ master of ship (name .....) (5) declare that the animals referred to in paragraph II above have remained on board the aircraft/ ship (5) during the flight/voyage (5) from ..... in Canada to ..... in the European Community and that the aircraft/ship (5) did not land/call (5) at any place outside Canada en route to the European Community.

Done at ....., on .....
(Port or airport of arrival) (Date of arrival)

Seal

.....
(Signature of captain or master)

.....
(Name in capital letters and title)

VIII. **Statement concerning health control at importation**

I, the undersigned, official veterinarian responsible for the frontier post at

....., in .....
(Location) (Member State)

certify that the animals described in paragraph II above have undergone a health control (import control) on arrival in the territory of the Community in accordance with the provisions of Article 12 (1) and 2 of Council Directive 72/462/EEC and have been admitted/refused entry (5).

Done at ....., on .....
(Frontier post) (Date of health control)

Seal

.....
(Signature of official veterinarian)

.....
(Name in capital letters and title)

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(1) A health certificate may be issued only in respect of animals transported in a single ship or aircraft to the same consignee.

(2) This time limit precedes the date of isolation on the holding of origin.

(3) Option to be indicated by importing Member State.

(4) A period of at least 30 days shall elapse between the tests referred to in subparagraphs 7 and 10 except that in the case of the tuberculin test a period of at least 42 days must elapse between the first and second test.

(5) Delete as appropriate.

(6) Delete when Article 3 derogation has been granted.

ANNEX B

HEALTH CERTIFICATE (1)
for domestic animals of the porcine species for breeding or production intended for consignment to the European Economic Community

No .....

Exporting country : CANADA

Competent ministry : CANADIAN DEPARTMENT OF AGRICULTURE

Competent issuing authority : CANADIAN DEPARTMENT OF AGRICULTURE

I. **Number of animals :** .....
(In words)

II. **Identification of animals**

Number of animals	Sex	Breed	Age	Official marks, other marks or brands (State No and position)

III. **Origin of animals**

The animals have originated from the following herd/herds (5) :

Official mark or brand	Herd of origin (Address and province)

IV. **Destination of animals**

The animals will be sent

from .....
(Place of loading)

to .....
(Member State and place of destination)

entering the territory of the Community

at .....
(Community frontier post)

by : Aircraft (Flight No .....)

Ship (Name .....) (5)

on ....
(Expected date and time of arrival)

Name and address of consignor : .....

.....

Name and address of consignee : .....

.....

V. **Attestation of health**

I, the undersigned, being an official veterinarian designated by the competent authority of Canada, certify that after due enquiry and to the best of my knowledge and belief the animals described above meet the following requirements :

1. They have remained in the territory of Canada for at least six months prior to the date of loading or since birth ;

2. They have been identified by an officially approved mark or brand as indicated in paragraph II above ;

3. They are not animals which are to be destroyed under a contagious or infectious-disease eradication programme ;

4. (i) They have not been vaccinated against porcine brucellosis, Aujeszky's disease, or swine influenza,

   (ii) They have/have not been vaccinated against Leptospirosis [3] [5] ;

5. They originate from herds :

   (i) which are recognized by the central veterinary authorities of Canada as being brucellosis-free herds,

   (ii) in which, immediately prior to the isolation period referred to in subparagraph 6, no evidence has been recorded which leads to a conclusion that

   — anthrax has occurred during the previous 30 days,

   — rabies, Aujeszky's disease, swine dysentery, viral encephalomyelitis (vomiting-and-wasting disease), leptospirosis and atropphic rhinitis have occurred during the previous six months,

   — transmissible gastroenteritis and swine influenza have occurred during the previous 12 months,

   (iii) which within the previous six months, have given negative results to a 10 % blood sample of adult breeding animals subjected to the serum neutralization test for transmissible gastroenteritis and/or the haemagglutination inhibition test for swine influenza [2] [3] [5] ;

6. They have, following a clinical examination at which they were found to be free of symptoms of communicable disease, been isolated from other animals in their herd of origin for a period of not less than 21 days ;

7. They have, during the 21-day isolation period referred to above been subjected to the following tests according to the provisions of Annex C of Commission Decision 83/494/EEC with negative results, the tests where appropriate having been performed at an officially approved laboratory :

   (i) the single intradermal tuberculin test for avian tuberculosis,

   (ii) the serum agglutination test and the complement fixation test for brucellosis,

   (iii) the serum neutralization test for Aujeszky's disease,

   (iv) the serum neutralization test for transmissible gastroenteritis,

   (v) the haemagglutination inhibition test for swine influenza,

   (vi) either the micro-agglutination test for leptospirosis (types L. pomona, grippotyphosa, hardjo and tarassovi (Hyos)) ; and/or they have, during the quarantine period referred to in subparagraph 10, and after completion of the tests referred to therein with negative results, with the exception of tests for leptospirosis, received an injection of dihydrostreptomycin (25 mg per kilogram of live body weight) [3] [5] ;

8. They have following the completion of the tests [4] laid down in subparagraph 7 above and of the 21-day isolation period been examined and found to be free from symptoms of communicable disease and that they show no evidence of ectoparasites ;

9. That, within 24 hours following the examination referred to in subparagraph 8 above they have been transported directly to the pre-embarkation quarantine premises referred to at subparagraph 10 below without coming into contact with cloven-hoofed animals other than bovine animals and swine meeting the provisions of Commission Decision 83/494/EEC, in vehicles which have been cleansed and disinfected under the supervision of an official of the Canadian veterinary services ;

10. That they have been resident in a pre-embarkation quarantine premises officially approved and supervised by the Canadian veterinary services until the tests (4) referred to in subparagraph 7 have been repeated with negative results. Tests for leptospirosis have/have not been repeated (3) (5). These tests have not been commenced until the animals have been resident in the quarantine premises for at least 21 days and where appropriate have been performed at an officially approved laboratory. The tuberculin test was commenced on ..... (date of injection) ;

11. That following the expiry of the quarantine period and the completion of the tests referred to in subparagraph 10 above, they were, following a clinical examination at which they were found to be free of symptoms of communicable disease, transported directly to the place of loading for consignment to the European Community without coming into contact with cloven-hoofed animals other than bovine animals and swine meeting the provisions of Commission Decision 83/494/EEC, in vehicles which have been cleansed and disinfected under the supervision of an official of the Canadian veterinary services ;

12. That at the place of loading they were placed on board a ship or aircraft in accommodation which has been cleansed and disinfected under the supervision of an official of the Canadian veterinary services and that no cloven-hoofed animals other than bovine animals or swine meeting the provisions of Commission Decision 83/494/EEC have been loaded onto the said ship or aircraft ; where crates have been used they were constructed of new materials.

VI. **This certificate is valid for 21 days from the date of loading.**

Done at ....., on .....
(Date of loading)

Seal

.....
(Signature of official veterinarian)

.....
(Name in capital letters and title)

VII. **Declaration by captain of the aircraft or master of the ship**

I, the undersigned, captain of aircraft (flight No .....)/ master of ship (name .....) (5) declare that the animals referred to in paragraph II above have remained on board the aircraft/ship (5) during the flight/voyage (5) from ..... in Canada to ..... in the European Community and that the aircraft/ship (5) did not land/call (5) at any place outside Canada en route to the European Community.

Done at ....., on .....
(Port or airport of arrival) (Date of arrival)

Seal

.....
(Signature of captain or master)

.....
(Name in capital letters and title)

VIII. **Statement concerning health control at importation**

I, the undersigned, official veterinarian responsible for the frontier post at

....., in .....
(Location) (Member State)

certify that the animals described in paragraph II above have undergone a health control (import control) on arrival in the territory of the Community in accordance with the provisions of Article 12 (1) and 2 of Council Directive 72/462/EEC and have been admitted/refused entry (5).

Done at ....., on .....
(Frontier post) (Date of health control)

Seal

.....
(Signature of official veterinarian)

.....
(Name in capital letters and title)

---

(1) A health certificate may be issued only in respect of animals transported in a single ship or aircraft to the same consignee.

(2) This time limit precedes the date of isolation on the holding of origin and such herd tests may be required by certain Member States.

(3) Option to be indicated by importing Member State.

(4) A period of at least 30 days shall elapse between the tests referred to in subparagraphs 7 and 10 except that in the case of the tuberculin test the period shall be at least 42 days.

(5) Delete as appropriate.

ANNEX C

I. Protocols for standardization of materials and testing procedures for bovine animals as referred to in Annex A V (7) and (10)

1. *Tuberculosis*

The single intradermal tuberculin test using bovine tuberculin shall be carried out according to Annex B to Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra Community trade in bovine animals and swine (1), as last amended by Council Directive 82/893/EEC (2).

2. *Brucellosis*

The serum agglutination test and complement fixation test shall be carried out according to paragraphs A and B of Annex C to Council Directive 64/432/EEC.

3. *Enzootic Bovine Leukosis*

The agar gel immuno-diffusion test shall be carried out according to Annex G to Council Directive 64/432/EEC.

4. *Blue tongue*

The agar gel immuno-diffusion test shall be carried out according to the following protocol :

(i) Antigen :

Precipitating antigen should be prepared in any cell culture system that supports the rapid multiplication of a reference strain of Blue tongue virus. BHK or Vero cells are recommended. Antigen is present in the supernatant fluid at the end of virus growth but requires 50 to 100-fold concentration to be effective. This may be achieved by any standard protein concentration procedure ; virus in the antigen may be inactivated by the addition of 0,3 % (V/V) Beta-propiolactone.

(ii) Test serum.

(iii) Known positive control serum :

should be standardized for optimal proportion against the international reference serum. Using the international reference antiserum and antigen a national standard serum should be produced, freeze-dried and used as the known positive control serum in each test.

(iv) Agar-gel :

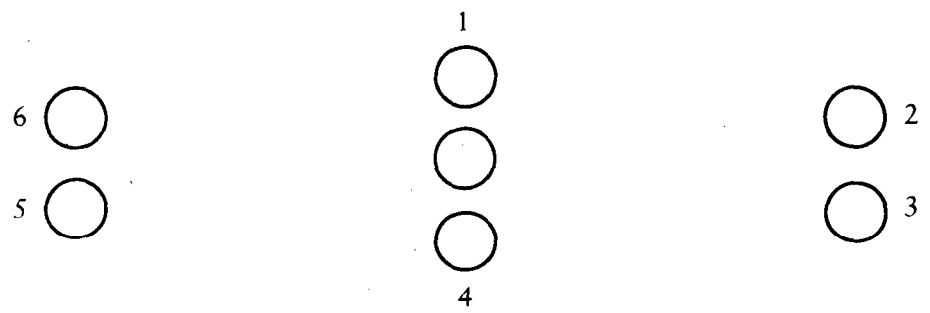
1 % agarose prepared in borate or sodium barbitol buffer pH 8,5 to 9,0. This is poured into a petri dish resulting in a minimum depth of 3,0 mm of agarose.

(v) A test pattern of seven moisture-free wells is cut in the agar ; the pattern consists of one centre well and six wells in a circle around it.

Diameter of wells : 5 mm.

Distance between central and peripheral well : 3 mm.

(vi) The central well is filled with standard antigen. The peripheral wells 2, 4 and 6 are filled with known positive serum ; the wells 1, 3 and 5 are filled with test sera.



(1) OJ No 121, 29. 7. 1964, p. 1977/64.
(2) OJ No L 378, 31. 12. 1982, p. 57.

(vii) Incubate for 72 hours at room temperature in a closed humid chamber. A test serum is positive if it forms a specific precipitin line with the antigen and forms a complete line of identify with the control serum. A test serum is negative if it does not form a specific line with the antigen and it does not bend the line of the control serum. Petri dishes should be examined against a dark background using indirect illumination.

5. *Epizootic haemorrhagic disease*

The agar gel immuno-diffusion test shall be carried out according to the following protocol :

(i) Antigen :

Precipitating antigen should be prepared in any cell culture system that supports the rapid multiplication of epizootic haemorrhagic disease virus (New Jersey strain). BHK or Vero cells are recommended. Antigen is present in the supernatant fluid at the end of virus growth but requires 50 to 100-fold concentration to be effective. This may be achieved by any standard protein concentration procedure ; virus in the antigen may be inactivated by the addition of 0,3 % (V/V) Beta-propiolactone.

(ii) Test serum.

(iii) Known positive control serum :

should be standardized for optimal proportion against the international reference serum. Using the international reference antiserum and antigen a national standard serum should be produced, freeze-dried and used as the known positive control serum in each test.

(iv) Agar-gel :

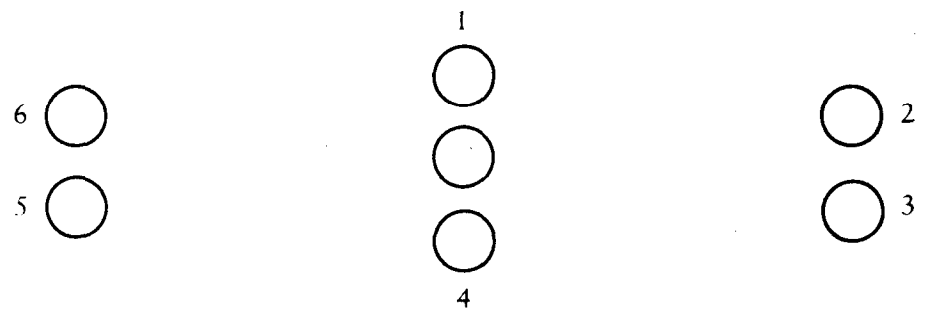
1 % agarose prepared in borate or sodium barbitol buffer pH 8,5 to 9,0. This is poured into a petri dish resulting in a minimum depth of 3,0 mm of agarose.

(v) A test pattern of seven moisture-free wells is cut in the agar ; the pattern consists of one centre well and six wells in a circle around it.

Diameter of wells : 5 mm.

Distance between central and peripheral well : 3 mm.

(vi) The central well is filled with standard antigen. The peripheral wells 2, 4 and 6 are filled with known positive serum ; the wells 1, 3 and 5 are filled with test sera.



(vii) Incubate for 72 hours at room temperature in a closed humid chamber. A test serum is positive if it forms a specific precipitin line with the antigen and forms a complete line of identity with the control serum. A test serum is negative if it does not form a specific line with the antigen and it does not bend the line of the control serum. Petri dishes should be examined against a dark background using indirect illumination.

6. *Leptospirosis*

The microscopic agglutination test shall be carried out according to the following protocol :

Cultures :

Are maintained in Korthof's and EMJH medium at 30 °C.

Antigen :

Should contain 2×10^8 organisms per ml of culture medium.

Test :

Equal amounts of antigen and serum to be used in flat-bottoned microtitre plates, mixed and incubated at 30 °C for two hours or 37 °C for one to one and a half hours and the test read by low-power dark-field illumination using a magnification between 60 and 100.

Interpretation :

A negative result is less than 50 % agglutination at a dilution of 1 : 100.

7. *Infectious bovine rhino-tracheitis/infections pustular vulvo-vaginitis*

The serum neutralization test shall be carried out according to the following protocol :

The constant virus-varying serum neutralization test should be performed using a microtitre test employing MDBK or other susceptible cells. The Colorado, Oxford or any other reference strain of the virus should be used at 100 $TCID_{50}$ per 0,025 ml ; inactivated undiluted serum samples are mixed with an equal volume (0,025 ml) of virus suspension. The virus/serum mixtures should be incubated for one hour at 37 °C in the microtitre plates before adding the MDBK cells. Cells are used at a concentration which forms a complete monolayer after 24 hours.

Serum :

All sera are heat-inactivated at 56 °C for 30 minutes before use.

Controls :

— virus infectivity assay,

— serum toxicity controls,

— uninoculated cell culture controls,

— reference antisera.

Interpretation :

The results of the neutralization test and the titre of the virus used in the text are recorded after three to six days incubation at 37 °C. Serum titres are considered negative if there is no neutralization at a dilution of 1 : 2 (undiluted serum).

8. *Bovine virus diarrhoea*

The serum neutralization test shall be carried out according to the following protocol :

The test should be performed in microtitration equipment by a constant virus-varying serum method which employs suitable serially propogated susceptible bovine cells (for example, bovine turbinate cells as described by McClurkin and others, 1974, Arch. ges. Virusforsch., 45, 285 — 289).

It is essential that all reagents and cells should be free from contaminating adventitious non-cytopathic BVD/MD virus. The test virus, which may be any suitable cytopathic reference strain (such as the NADL strain) is used at a concentration of 100 median cell culture infectious doses per 0,05 ml. Dilutions of inactivated sera are mixed with equal volumes of virus suspension (0,05 ml) and the virus-serum mixtures are incubated for one hour at 37 °C before similar volumes of cell suspension are added. The cells are used at a concentration which will form a complete monolayer within two days.

Sera are inactivated at 56 °C for 30 minutes before testing.

Adequate controls must be included :

Controls :

— virus infectivity assay,

— serum toxicity controls,

— uninoculated cell culture controls,

— reference antisera.

Reading and interpretation :

With strain NADL the optimum time for reading is after five days incubation at 37 °C.

A median neutralization titre of one-tenth is considered to be indicative of an immune response to past acute infection.

9. *Milk analysis for mastitis*

Milk analysis shall be carried out according to Annex D to Council Directive 64/432/EEC, as last amended by Council Directive 82/893/EEC.

II. Protocols for standardization of materials and testing procedures for porcine animals as referred to in Annex B V (7) and (10)

1. *Tuberculosis*

The single intradermal tuberculin test using avian tuberculin shall be carried out according to Annex B to Council Directive 64/432/EEC, as last amended by Directive 82/893/EEC, except that the site of injection shall be the loose skin at the base of the ear.

2. *Brucellosis*

The serum agglutination test and complement fixation test shall be carried out according to paragraphs A and B of Annex C to Council Directive 64/432/EEC, as last amended by Directive 82/893/EEC.

3. *Aujesky's disease*

The serum neutralization test shall be carried out according to the following protocol :

The constant virus-varying serum neutralization test should be performed using a microtitre test employing Vero cells or other sensitive cell systems. Aujeszky's disease virus should be used at 100 $TCID_{50}$ per 0,025 ml ; inactivated undiluted serum samples are mixed with an equal volume (0,025 ml) of virus suspension. The virus/serum mixtures should be incubated for one hour at 37 °C in the microtitre plates before adding the appropriate cells. Cells are used at a concentration which forms a complete monolayer after 24 hours. Each well receives 0,05 ml of cell suspension.

Serum :

All sera are heat-inactivated at 56 °C for 30 minutes before use.

Controls :

— virus infectivity assay,
— serum toxicity controls,
— uninoculated cell culture controls,
— reference antisera.

Interpretation :

The results of the neutralization test and the titre of the virus used in the text are recorded after three to seven days incubation at 37 °C. Serum titres less than one in two are considered negative.

4. *Transmissible gastroenteritis*

The serum neutralization test shall be carried out according to the following protocol :

The constant virus-varying serum neutralization test is used. Serum/virus mixtures are inoculated onto early confluent monolayer cultures of primary pig kidney cells or other sensitive pig cell systems. TGE virus should be used at 100 $TCID_{50}$ per volume ; inactivated (undiluted) serum samples are mixed with an equal volume of virus suspension. The virus/serum mixtures should be incubated for 30 to 60 minutes at 37 °C and then inoculated onto suitable primary pig kidney cells or other susceptible pig cell line.

Serum : All sera are heat-inactivated at 56 °C for 30 minutes.

Controls :

— virus infectivity assay,
— serum toxicity controls,
— uninoculated cell culture controls,
— reference antisera.

Interpretation :

The results of the neutralization test and the titre of the virus used in the test are recorded after five days incubation at 37 °C. Serum titres less than one in two are considered negative. With the micro titre test serum titres less than one in four are considered negative.

5. *Swine influenza*

The haemagglutination-inhibition test shall be carried out according to the following protocol :

These tests are performed by standard methods (US Department of Health, Education and Welfare, Immunology series No 6) using the A/Swine/Wisconsin/15/30 and A/Swine/Belgium/1/79 (H_1N_1) strains. To destroy unspecific inhibitors swine sera should be treated with either receptor destroying enzyme (Vibrio cholerae filtrate) overnight at 37 °C followed by heating at 56 °C for 30 minutes to destroy residual enzyme activity, or by treating with 25 % kaolin overnight at 4 °C (Clarke and Casals 1958, American Journal for Tropical Medicine and Hygiene, 7, 561).

After absorption with a 10 % suspension of chicken erythrocytes for one hour at 37 °C, sera are tested against four haemagglutinating units of virus using 1 % chicken erythrocytes. Virus and serum should be left in contact for 60 minutes at room temperature before adding erythrocytes.

Titres of one-tenth or greater are considered positive.

6. *Leptospirosis*

The microscopic agglutination test shall be carried out according to the following protocol :

Cultures :

Are maintained in Korthof's and EMJH medium at 30 °C.

Antigen :

Should contain 2×10^8 organisms per ml of culture medium.

Test :

Equal amounts of antigen and serum to be used in flat-bottomed microtitre plates, mixed and incubated at 30 °C for two hours or 37 °C for one to one and a half hours and the test read by low-power dark-field illumination using a magnification between 60 and 100.

Interpretation :

A negative result is less than 50 % agglutination at a dilution of 1 : 100.