

**VETERINARY SERVICES ACT  
(CAP. 437)**

**Animal Health Problems Affecting Intra-Community Trade in Bovine Animals and Swine Rules, 2004**

IN exercise of the powers conferred by article 8 of the Veterinary Services Act, 2001, the Minister for Rural Affairs and the Environment has made the following rules:-

**Title, scope and applicability**

1. (1) The title of these regulations is Animal Health Problems Affecting Intra-Community Trade in Bovine Animals and Swine rules, 2004.

(2) The scope of these rules is to implement the rules laid down under European Union Council Directive 64/432/EEC on animal health problems affecting intra-Community trade in bovine animals and swine:

Provided that for the purposes of these regulations any reference made under European Union Council Directive 64/432/EEC to Annex F has now been harmonised under Commission Regulation (EC) No 599/2004 of the 30<sup>th</sup> March 2004 concerning the adoption of a harmonised model certificate and inspection report linked to intra-Community trade in animals and products of animal origin:

Provided further the certificate found under commission decision 599/2004 shall apply to these rules.

(3) These rules shall apply to intra-Community trade in bovine animals and swine with the exception of feral pigs as defined in Article 2 (e) of the European Union Council Directive 80/217/EEC without prejudice to the provisions laid down in the European Union Council Directives 80/215/EEC, 85/511/EEC, 88/407/EEC, 89/608/EEC, 90/425/EEC, 90/429/EEC, 90/667/EEC, 91/496/EEC, 91/628/EEC, 92/102/EEC, 92/119/EEC and Decision 90/424/EEC.

**Definitions**

2. (1) (a) The definitions given in article 2 of European Union Council Directive 90/425/EEC relating to veterinary checks applicable in trade in bovine and porcine animals and in article 2 of European Union Council Directive 91/628/EEC relating to animal welfare during transport shall apply to these regulations.

(b) “Member States” means Member States of the European Union;

“Third Countries” means countries which are not Member States

within the European Union;

(2) In addition, the following definitions apply for the purposes of these regulations -

“animals for breeding or production” means bovine animals (including the species *Bison bison* and *Bubalus bubalus*) and swine other than those referred to as animals for slaughter, including those intended for breeding, milk or meat production, or draft purposes, shows or exhibition with the exception of animals taking part in cultural and sporting events;

“animal for slaughter” means a bovine animal (including the species *Bison bison* and *Bubalus bubalus*) or swine intended to be taken to a slaughterhouse or assembly centre from which it may only move to slaughter;

“approved veterinarian” means any veterinarian approved by the Veterinary Services, in accordance with the provisions of rule 14 (3) (B) ;

“assembly centre” means holdings, collection centres and markets, at which bovine animals or swine originating from different holdings are grouped together to form consignments of animals intended for trade. These assembly centres must be approved for trading purposes and meet the requirements laid down in rule 11;

“brucellosis-free bovine herd” means a bovine herd which satisfies the conditions laid down in Annex A. II, paragraphs 4, 5 and 6 to European Union Council Directive 64/432/EEC;

“competent authority” means –

(i) for Malta: the Veterinary Services as established under article 2 of the Veterinary Services Act;

(ii) for a Member State: the central authority competent to carry out veterinary checks in animal health or any authority to which it has delegated that competence;

“compulsorily notifiable diseases” means the diseases listed in Annex E (I) to European Union Council Directive 64/432/EEC;

“dealer” means any natural or legal person who buys and sells animals commercially either directly or indirectly, who has a regular turnover of these animals and who within a maximum of 30 days of purchasing animals resells them or relocates them from the first premises to other premises not within his ownership and meets the conditions laid down in rule 13;

“herd” means an animal or group of animals kept on a holding (within the meaning of Article 2 (b) of Directive 92/102/EEC) as an epidemiological unit; if more than one herd is kept on a holding, each of these herds shall form a distinct unit and shall have the same health status;

“officially brucellosis free bovine herd” means a bovine herd which satisfies the conditions laid down in Annex A. II, paragraphs 1, 2 and 3 to European Union Council Directive 64/432/EEC;

“officially brucellosis free Member State” means a Member State which satisfies the conditions laid down in Annex A. II, paragraphs 10, 11 and 12 to European Union Council Directive 64/432/EEC;

“officially brucellosis free region” means a region of a Member State which satisfies the conditions laid down in Annex A. II, paragraphs 7, 8 and 9 to European Union Council Directive 64/432/EEC;

“officially enzootic-bovine-leukosis-free herd” means a herd which satisfies the conditions laid down in Annex D, Chapter I, Sections A and B, C and D to European Union Council Directive 64/432/EEC;

“officially enzootic-bovine-leukosis-free Member State or region” means a region or Member State which meets the requirements laid down in Annex D, Chapter I, Sections E and F, and G to European Union Council Directive 64/432/EEC;

“officially tuberculosis free bovine herd” means a bovine herd which satisfies the conditions laid down in Annex A. I, paragraphs 1, 2 and 3 to European Union Council Directive 64/432/EEC;

“officially tuberculosis free Member State or region of a Member State” means a Member State or part of a Member State which satisfies the conditions laid down in Annex A. I, paragraphs 4, 5 and 6 to European Union Council Directive 64/432/EEC;

“official veterinarian” means the veterinarian appointed by the competent authority of a Member State;

“region” means that part of a Member State's territory which is subject to inspection by the competent authorities.

(i) Belgium: *province/provincie*;

(ii) Germany: *Regierungsbezirk*;

(iii) Denmark: *amt* or *island*;

- (iv) France: *department*;
- (v) Italy: *provincial*;
- (vi) Luxemburg Netherlands: *rrv-kring*;
- (vii) United Kingdom - England, Wales and Northern Ireland: *county*;
- (viii) Scotland: *district or island area*;
- (ix) Ireland: *county*;
- (x) Greece: *Nomos*;
- (xi) Spain: *provincial*;
- (xii) Portugal: *continente: distrito*, and other parts of Portugal's territory: *região autónoma*;
- (xiii) Austria: *Bezirk*;
- (xiv) Sweden: *län*;
- (xv) Finland: *lääni/län*.

### **Conditions to be fulfilled by animals for export**

**3.** (1) Only animals that fulfil the relevant conditions laid down in these rule are sent from the Maltese territory to that of another Member State.

(2) Bovine animals and swine covered by these rule must -

(a) be subjected -

(i) to an identity check, and

(ii) to a clinical inspection within 24 hours of departure by an official veterinarian and show no clinical sign of disease;

(b) not have been obtained from a holding or an area which for health reasons is subject to prohibition or restriction affecting the species involved in accordance with Community and or national legislation;

(c) be identified as provided for in European Union Council Directive 92/102/EEC

(d) not be animals which are to be slaughtered and not be restricted under

a contagious or infectious disease eradication programme in Malta;

(e) comply with the provisions of rules 4 and 5.

#### **Contact between animals**

4. (1) Bovine animals and swine covered by these regulations must at no time between leaving the holding of origin and arriving at destination come into contact with cloven-hoofed animals other than animals that have the same health status.

(2) Bovine animals and swine covered by these regulations must be transported by means of transport meeting the requirements of European Union Council Directive 91/628/EEC and in addition the requirements of rule 12.

(3) Rules for the approval of sites where cleansing and disinfection may be carried out shall be determined in accordance with the procedure set out in Article 17 of the European Union Council Directive 64/432/EEC .

#### **Health certificate to accompany animals during transportation**

5. (1) Bovine animals and swine covered by these rule smust be accompanied during transportation to destination by a health certificate conforming to the model 1 or 2 set out in Annex F as appropriate. The certificate shall consist of a single sheet or, where more than one page is required, shall be in such a form that any two or more pages are part of an integrated whole and indivisible and shall contain a serial number. It shall be drawn up on the day of the health inspection, in one of the official language, of the country of destination at least. The certificate shall be valid for 10 days from the date of the health examination.

(2) The health inspection for the issuing of the health certificate (including additional guarantees) for a consignment of animals may be carried out in the holding of origin or of an assembly centre. For this purpose the Veterinary Services shall ensure that any certificate is drawn up by the official veterinarian after inspections, visits and controls as provided by these rules. However as regards -

(a) animals coming from approved assembly centres such certification shall be -

(i) on the basis of an official document containing the necessary information completed by the official veterinarian for the holding of origin, or

(ii) in the form of the certificate according to either model 1 or 2 in Annex F as appropriate with Sections A and B duly completed and certified by the official veterinarian for the holding of origin;

(b) animals coming from an approved holding which is participating in the surveillance network provided for in regulation 14, such certification shall be:

(i) on the basis of an official document containing the necessary information completed by the approved veterinarian for the holding of origin, or

(ii) in the form of the certificate according to either model 1 or 2 in Annex F as appropriate with Sections A and B duly completed and certified by the approved veterinarian for the holding of origin. For this purpose, the official veterinarian will ensure where appropriate that the additional guarantees provided for in Community legislation are fulfilled.

(3) The official veterinarian for the assembly centre shall carry out all necessary checks on animals arriving there.

(4) The official veterinarian completing Section C of the certificate according to either model 1 or 2 in Annex F as appropriate, shall ensure that the movement is registered on the Animo system on the day the certificate is issued .

(5) The animals covered by these regulations may transit through an assembly centre which is located in one other Member State before being consigned to the Member State of destination. In this case, the certificate according to either model 1 or 2 in Annex F as appropriate (including Section C) must be completed by the official veterinarian responsible in the Member states where the animals originate. The official veterinary responsible for the assembly centre of transit shall provide certification to the member state of destination by completing a second certificate according to either model 1 or 2 in Annex F as appropriate, endorsing it with the serial number of the original attaching it to the original certificate or to an officially endorsed copy thereof. In this case the combined validity of the certificates shall not exceed that provided for in sub- rule (1).

#### **Further requirements for animals for breeding or production**

**6.** (1) Animals for breeding or production must, in addition to the requirements of rules 3, 4 and 5 -

(a) have remained in a single holding of origin for a period of 30 days prior to loading, or since birth in the holding of origin where the animals are less than 30 days old. The official veterinarian must, on the basis of the official identification provided for in regulation 3 (2) (c) and official records, be satisfied that the animals have complied with this condition and furthermore that the animals have originated in Malta or in the European Community or have been imported from a third country in

compliance with European Community animal health legislation:

Provided that in the case of animals transiting through an approved assembly centre in Malta or in the Member State of origin, the period during which the assembly of these animals takes place outside the holding of origin shall not exceed six days;

(b) with regard to animals imported from a third country into the territory of Malta which is not the ultimate destination, be transported to the Member State of destination as quickly as practicable under cover of a certificate issued under Article 7 of European Union Council Directive 91/496/EEC;

(c) with regard to animals imported from a third country upon arrival in the territory of Malta and before any further movements, satisfy the requirements of these regulations, and in particular the residency requirement in sub-rule (1)(a), and may not be brought into the herd until the veterinarian responsible for that holding has ascertained that the animals in question are not likely to jeopardise the health status of the holding:

Provided that if an animal from a third country is introduced into a holding no animal from the holding may be traded for 30 days following introduction unless the imported animal is isolated from all other animals on the holding.

(2) Bovine animals for breeding and production must, in addition to the requirements in rule 3, 4 and 5 -

(a) come from an officially tuberculosis-free bovine herd, and in the case of animals more than six weeks old, have reacted negatively to an intradermal tuberculin test carried out during the 30 days prior to leaving the herd of origin, in accordance with the provisions of Schedule B point 32 (d):

Provided that this intradermal tuberculin test is not required if the animals originate in a Member State or part of a Member State recognised as officially tuberculosis free or in a Member State or part of a Member State with an approved surveillance network;

(b) in the case of uncastrated animals which come from an officially brucellosis free bovine herd and more than 12 months old, have shown a brucella count lower than 30 international units (IU) of agglutination per millilitre when given a serum agglutination test (or any test approved by Standing Veterinary Committee (SVC) procedure following the adoption of the relevant protocols) carried out during the 30 days prior to the leaving

the herd of origin and complying with the provisions of Annex C Section 2.2.2.1. (a):

Provided that this serum agglutination test (or any test approved by SVC procedure following the adoption of the relevant protocols) is not required if the animals originate in a Member State or part of a Member State recognized as officially brucellosis free or in a Member State or part of a Member State with an approved surveillance network;

(c) come from an officially enzootic-bovine-leukosis-free herd and, if more than 12 months old, have reacted negatively to an individual test carried out during the 30 days prior to leaving the herd of origin and complying with the provisions of Schedule D:

Provided that the test is not required if the animals originate in a Member State or part of a Member State recognised as officially enzootic-bovine-leukosis free or in a Member State or part of a Member State with an approved surveillance network;

(d) at no time between leaving the holding of origin and arriving at destination come into contact with bovine animals which meet only the requirements in sub- rule (3);

(e) until 31 December 2000, not be subject to the test requirements laid down in paragraph (a) or (b) in the case of bovine animals aged less than 30 months intended for meat production which-

(i) come from a beef holding officially tuberculosis-free and officially brucellosis-free;

(ii) are accompanied by an animal health certificate with paragraph 7 in Section A of Annex F Model 1 duly completed;

(iii) remain under supervision until their slaughter;

(iv) have not come into contact during transport with bovine animals not coming from herds officially free from those diseases.

and provided that-

(i) these arrangements are restricted to trade between Member States or regions of Member States with the same health status with regard to tuberculosis or brucellosis;

(ii) the Member State of destination takes all necessary measures to avoid any contamination of indigenous herds;

(iii) the Member States put in place a proper system of random sampling, inspections and controls designed to ensure the efficient implementation of these rules;

(iv) the Commission monitors the proper operation of this Directive so as to ensure that Member States comply fully with the rules.

(3) Bovine animals for slaughter must, in addition to the requirements in rules 3, 4 and 5, come from herds that are officially tuberculosis free, officially enzootic-bovine-leukosis free and in the case of uncastrated bovines, from herds that are officially brucellosis free.

#### **Animals for slaughter**

7. Animals for slaughter which have been taken on arrival in the territory of Malta

(a) to a slaughterhouse, must be slaughtered there as soon as possible but at least within 72 hours of arrival, in accordance with animal health requirements, or

(b) to an approved assembly centre, must be removed directly to a slaughterhouse to be slaughtered as soon as possible but at the latest within three working days of arrival at the assembly centre, in accordance with animal health requirements. At no time, between their arrival at the assembly centre and their arrival at the slaughterhouse, may they come into contact with cloven-hoofed animals other than animals that fulfil the conditions laid down in these rules.

#### **Immediate notification of suspected disease**

8. The suspected presence of any of the diseases referred to in Schedule E (I) of these regulations is compulsorily and immediately notifiable to the competent authority.

The competent authority shall forward to the European Commission by 31 May each year, and for the first time in 2004, details of the occurrence of diseases listed in Schedule E (1) of these rules and of any other diseases covered by the additional guarantees provided for by European Community legislation in the territory of Malta in the previous calendar year including details of the monitoring and eradication programmes in operation. This information shall be based on uniform criteria established by European Commission. The Commission shall present this information to the Member States in the framework of the SVC and in particular may utilize it in relation to the decisions referred to in Schedules A and D.

**Compulsory national control programme for contagious diseases**

9. (1) On the basis of a Veterinary services proposal, the Minister in charge of Agriculture may define, by virtue of implementary regulation a compulsory national control programme for one of the contagious diseases listed in Annex E (II) to Directive 64/432/EEC for all of the territory of Malta. The said programme shall be submitted to the European Commission and outlined in particular:

- (a) the distribution of the disease in the Member State,
- (b) the reasons for the programme, taking into consideration the importance of the disease and the programme's likely benefit in relation to its cost,
- (c) the geographical area in which the programme will be implemented,
- (d) the status categories to be applied to the animal establishments, the standards which must be attained in each category, and the test procedures to be used,
- (e) the programme monitoring procedures, the results of which must be supplied at least annually to the Commission,
- (f) the action to be taken if, for any reason, an establishment loses its status,
- (g) the measures to be taken if the results of the tests carried out in accordance with the provisions of the programme are positive.

(2) The Commission shall examine the programmes presented by the Member States. Programmes as referred to in sub-rule (1) may be approved in compliance with the criteria laid down in paragraph 1 in accordance with the procedure provided for in Article 17 of the European Union Council Directive 64/432/EEC. According to the same procedure, the additional guarantees, general or limited, which may be required in intra-Community trade, shall be defined at the same time or at the latest three months after approval of the programmes. Such guarantees must not exceed those which the Member State implements nationally.

(3) Programmes submitted by Member States may be amended or supplemented in accordance with the procedure laid down in Article 17 of the European Union Council Directive 64/432/EEC. Amendments or additions to programmes which have already been approved or to guarantees which have been defined in accordance with sub-rule (2) may be approved under the same procedure.

(4) The dispatching of pigs intended for breeding or production destined for the Member States or regions free of Aujeszky's disease listed in Annex I of Commission

Decision 2001/618/EC and coming from the territory of Malta or region not listed in that Annex is authorised subject to the following conditions:

(a) Aujeszky's disease must be compulsorily notifiable in Malta or region of origin;

(b) a plan for the control and eradication of Aujeszky's disease, fulfilling the criteria laid down in the first paragraph of this Article, must be in place in Malta or regions of origin under the supervision of the Veterinary Services. Appropriate measures on pig transport and movements must be in place according to this plan for preventing a spread of disease between holdings of a different status;

(c) with regard to the holding of origin of the pigs:

(i) no clinical, pathological or serological evidence of Aujeszky's disease has been recorded in the previous 12 months in the holding in question;

(ii) no clinical, pathological or serological evidence of Aujeszky's disease has been recorded in the previous 12 months in the holdings located in an area of 5 km surrounding the holding of origin of the pigs; however, this provision shall not apply if, in these latter holdings, disease monitoring and eradication measures have been regularly applied under the supervision of the Veterinary Services and in accordance with the eradication plan referred to in point (b), and these measures have effectively prevented any spread of disease to the holding in question;

(iii) vaccination against Aujeszky's disease has not been carried out for at least 12 months;

(iv) the pigs have been subjected on at least two occasions at a distance of at least four months to a serological survey for the presence of ADV-gE or ADV-gB or ADV-gD antibody or to the whole Aujeszky's disease virus. This survey must have shown the absence of Aujeszky's disease and that vaccinated pigs have been free from gE antibodies;

(v) no pigs have been introduced from holdings of a lower animal health status as regards Aujeszky's disease in the previous 12 months, unless they have been tested for Aujeszky's disease with negative results;

(d) the pigs to be moved:

- (i) have not been vaccinated;
- (ii) have been kept isolated in accommodation approved by the Veterinary Services, during the 30 days prior to movement, and in such a way that any risk of spreading Aujeszky's disease to these pigs is prevented;
- (iii) must have lived in the holding of origin or in a holding of an equivalent status since birth, and have remained in the holding of origin for at least:
  - 30 days, in the case of pigs intended for production;
  - 90 days, in the case of pigs intended for breeding, have been subjected with negative results to at least two serological tests for ADV-gB or ADV-gD or the whole Aujeszky's disease virus, at a distance of at least 30 days between each test. However, in case of pigs less than four months old, the serological test for ADV-gE may also be used. Sampling for the last test must be performed within 15 days prior to shipment. The number of pigs tested in the isolation unit must be sufficient to detect:
    - (i) 2 % seroprevalence with 95 % confidence in the isolation unit in case of pigs intended for production;
    - (ii) 0,1 % seroprevalence with 95 % confidence in the isolation unit in case of pigs intended for breeding.

However, the first of the two tests shall not be necessary if:

- (i) in the framework of the plan referred to in point (b), a serological survey has been carried out in the holding of origin between 45 and 17 days prior to shipment, demonstrating the absence of Aujeszky's disease antibodies and that vaccinated pigs have been free from gE antibodies;
- (ii) the pigs to be moved have lived in the holding of origin since birth;
- (iii) no pigs have moved on to the holding of origin while the pigs to be moved have been kept in isolation.

(5) The dispatching of pigs intended for slaughter destined for the Member States or regions free of Aujeszky's disease listed in Annex I of Commission Decision 2001/618/EC and coming from Malta or region not listed in that Annex, is authorised subject to the following conditions:

(a) Aujeszky's disease must be compulsorily notifiable in Malta or region of origin;

(b) a plan for the control and eradication of Aujeszky's disease is in place in Malta or regions of origin of the pigs, fulfilling the criteria laid down under European Union Law;

(c) all the pigs in question must be transported directly to the slaughterhouse of destination and either:

(i) they come from a holding which fulfils the conditions required by European Union Law, or

(ii) they have been vaccinated against Aujeszky's disease at least 15 days prior to their shipment and come from a holding of origin where:

-in the framework of the plan referred to in point (b), Aujeszky's disease monitoring and eradication measures have been regularly applied under the supervision of the Veterinary Services for the previous 12 months;

- they had remained for at least 30 days before dispatch and where no clinical or pathological evidence of this disease has been detected at the moment of completion of the health certificate referred to in rule 5, or

- they have not been vaccinated and they proceed from a holding where:

(i) in the framework of the plan referred to in point (b), Aujeszky's disease monitoring and eradication measures have been regularly applied under the supervision of the Veterinary Services in the previous 12 months and no clinical, pathological or serological evidence of Aujeszky's disease has been recorded in the previous six months;

(ii) vaccination against Aujeszky's disease and introduction of vaccinated pigs have been forbidden by the Veterinary Services, since the holding is in the process of reaching the highest status as regards Aujeszky's disease in accordance to the plan referred to in point (b);

(iii) they have lived for at least 90 days before dispatch.

(6) Pigs intended for breeding destined for the Member States or regions listed in Annex II of Commission Decision 2001/618/EC, where approved Aujeszky's disease eradication programmes are in place, must either:

(a) come from Member States or regions listed in Annex I of Commission Decision 2001/618/EC, or

(b) come from:

(i) Member States or regions listed in Annex II, and

(ii) a holding which fulfils the requirements of paragraph 3 (c); or

(c) fulfil the following conditions:

(i) Aujeszky's disease must be compulsorily notifiable in Malta or region where pigs are originating,

(ii) a plan for the control and eradication of Aujeszky's disease is in place in Malta or region of origin, which fulfils the criteria laid down in paragraph 3 (b),

(iii) no clinical, pathological or serological evidence of Aujeszky's disease has been recorded in the previous 12 months in the holding of origin of the pigs in question,

(iv) the pigs must have been isolated in accommodation approved by the Veterinary Services for the 30 days immediately prior to movement and kept isolated in such a way that any risk of spreading of Aujeszky's disease is prevented,

(v) the pigs must have been subjected, with negative results, to a serological test for the presence of gE antibodies. Sampling for the last test must be performed within 15 days prior to shipment. The number of pigs tested must be sufficient to detect 2 % seroprevalence with 95 % confidence in these pigs,

(vi) the pigs must have lived in the holding of origin or in a holding of an equivalent status since birth, and have remained in the holding of origin for at least 90 days.

(7) Pigs intended for production destined for the Member States or regions listed in Annex II Commission Decision 2001/618/EC, where approved Aujeszky's disease eradication programmes are in place, must either:

(a) come from Member States or regions listed in Annex I Commission Decision 2001/618/EC, or

(b) come from:

(i) Member States or regions listed in Annex II Commission Decision 2001/618/EC, and

(ii) a holding which fulfils the requirements of sub regulation (3) (c) of this regulation; or

(c) fulfil the following conditions-

(i) Aujeszky's disease must be compulsorily notifiable in Malta or region of origin,

(ii) a plan for the control and eradication of Aujeszky's disease is in place in Malta or region of origin, which fulfils the criteria laid down in sub regulation (3) (b) of this regulation;

(iii) no clinical, pathological or serological evidence of Aujeszky's disease has been recorded in the previous 12 months in the holding of origin of the pigs in question;

(iv) a serological survey for Aujeszky's disease, demonstrating its absence and that vaccinated pigs have been free from gE antibodies, has been carried out in the holding of origin and between 45 and 170 days prior to shipment;

(v) the pigs must either have lived in the holding of origin since birth or have remained in such holdings for at least 30 days after introduction from a holding of an equivalent status, where a serological survey equivalent to the one referred to in the fourth indent above has been carried out;

(8) The serological tests carried out to monitor or detect Aujeszky's disease in pigs in accordance with these regulations must meet the standards laid down in Annex III to the European Union Commission Decision 2001/618/EC.

(9) Without prejudice to Article 10(3) of Directive 64/432/EEC, information of the occurrence of Aujeszky's disease, including details of the monitoring and eradication programmes in operation in Malta where monitoring and eradication programmes will be in place, must be provided at least annually by the Veterinary Services in accordance with the uniform criteria laid down in Annex IV to Commission Decision 2001/618/EC.

(10) Without prejudice to the provisions laid down in Community legislation concerning health certificates, before the completion, for animals of the porcine species destined for Member States or regions listed in Annex I or II to Commission Decision 2001/618/EC, of section C of the health certificate required by Directive 64/432/EC, the official veterinarian shall ascertain:

(a) the status of the holding and of the Member State or region of origin of the pigs in question as regards Aujeszky's disease;

(b) in case the pigs are not originating from a Member State or a region free of the disease, the status of the holding and of the Member State or regions of destination for the pigs in question as regards Aujeszky's disease;

(c) the compliance of the pigs in question with the conditions laid down in Commission Decision 2001/618/EC.

(11) For animals of the porcine species destined for Member States or regions listed in Annex I or II, European Union Commission Decision 2001/618/EC, the certification under sub regulation 10 shall be completed and supplemented as follows:

(a) in the first indent, after the word "disease:" the word "Aujeszky" must be added;

(b) in the second indent, reference shall be made to Commission Decision 2001/618/EC. In the same line, the number of the Article of Commission Decision 2001/618/EC, which is relevant for the pigs in question, shall be quoted between brackets.

(12) The Veterinary Services must ensure that when pigs destined for Member States or regions listed in Annex I or II to Commission Decision 2001/618/EC, are transported, they shall not come in contact with pigs of different or unknown status, as regards Aujeszky's disease, during transport or transit.

**Territory free from the diseases listed in Annex E to EU Council Directive 64/432/EEC**

**10.** (1) Where the competent authority considers that the Maltese territory is free from one of the diseases listed in Annex E (II) of these rules, they shall present to the European Commission appropriate supporting documentation, setting out in particular-

(a) the nature of the disease and the history of its occurrence in its territory;

(b) the results of surveillance testing based on serological, microbiological, pathological or epidemiological investigation and on the fact that the disease must by law be notified to the competent authorities;

(c) the period over which the surveillance was carried out;

(d) where applicable, the period during which vaccination against the disease has been prohibited and the geographical area concerned by the prohibition;

(e) the arrangements for verifying the absence of the disease.

(2) The documentation submitted by the competent authority shall be examined by the European Commission. The additional guarantees, general or specific, which may be required in Intra-Community trade may be defined in accordance with Article 17 of the European Union Council Directive 64/432/EEC. Such guarantees must not exceed those which are implemented nationally.

(3) The competent authority shall notify the European Commission of any change in the details specified in sub-regulation (1) which relate to the disease, in particular regarding any new outbreaks of the disease. The guarantees defined in accordance with sub-regulation (2) may, in the light of such notification, be amended or withdrawn in accordance with the procedure laid down in Article 17 of the European Union Council Directive 64/432/EEC.

#### **Minimum conditions for assembly centres**

**11.** (1) To be approved by the competent authority, assembly centres meet the following conditions at least. They must -

(a) be under the control of an official veterinarian who shall ensure that, in particular, the provisions of rule 4 (1) and (2) are complied with;

(b) be located in an area which is not subject to prohibition or restrictions in accordance with relevant European Community legislation and, or Maltese rules on animal diseases;

(c) be cleaned and disinfected before use, as required by the official veterinarian;

(d) they must have, taking into account the animal capacity of the assembly centre -

(i) a facility dedicated exclusively for this purpose when used as an assembly centre;

(ii) appropriate facilities for loading, unloading and adequate housing of a suitable standard for the animals, for watering and feeding them, and for giving them any necessary treatment; these facilities must be easy to clean and disinfect;

- (iii) appropriate inspection facilities;
- (iv) appropriate isolation facilities;
- (v) appropriate equipment for cleaning and disinfecting rooms and trucks;
- (vi) an appropriate storage area for fodder, litter and manure;
- (vii) an appropriate system for collecting waste water;
- (viii) the use of an office for the official veterinarian;

(e) admit only animals that are identified and come from herds that are officially free of tuberculosis, brucellosis and leukosis or slaughter animals meeting the conditions set out in the present regulations and in particular rule 6 (3). To this end, when animals are admitted the owner or person in charge of the centre shall ensure they are properly identified and accompanied by health documents or appropriate certificates for the species and categories involved;

(f) be regularly inspected to ascertain that the requirements for approval continue to be fulfilled.

(2) The owner or person in charge of the assembly centre shall be required, on the basis either of the accompanying documents for the animals or of the identification numbers or marks of the animals, to record on a register or a data base and retain for a minimum period of three years the following information -

(a) the name of the owner, the origin, date of entry and exit, number and identification of the bovine animals or the registration number of the holding of origin or of the herd of origin of the pigs entering the centre and their proposed destination,

(b) the registration number of the transporter and the licence number of the lorry delivering or collecting animals from the centre.

(3) The competent authority shall issue an approval number to each approved assembly centre. Such approval may be limited to a particular species or to animals for breeding and production or to animals for slaughter. The competent authority shall notify the European Commission of the list of approved assembly centres and of any updates.

(4) The competent authority may suspend or withdraw approval in the event of failure to comply with this regulation or other appropriate provisions of these regulations or other regulations in respect of health restrictions. Approval may be restored when the

competent authority is satisfied that that assembly centre is in full compliance with all the appropriate provisions of these regulations.

(5) The competent authority shall ensure that when operating assembly centres have sufficient approved veterinarians to carry out all duties.

(6) Any detailed rules shall be adopted in accordance with this regulation shall be adopted requirements of the European Community and under the procedure provided for in Article 17 of the European Union Council Directive 64/432/EEC.

### **Additional conditions for transporters**

**12.** (1) The transporters referred to in Article 5 of European Union Council Directive 91/628/EEC meet the following additional conditions -

(a) for the carriage of animals they must use means of transport that are -

(i) constructed in such a way that the animal faeces, litter or feed cannot leak or fall out of the vehicle;

(ii) cleaned and disinfected immediately after every animal transport operation or that of any product which could affect animal health and if necessary before any new loading of animals, using disinfectants officially authorised by competent authority;

(b) they must either have appropriate cleaning and disinfection facilities approved by the competent authority, including facilities for storing litter and dung, or they must provide documentary evidence that these operations are performed by a third party approved by the competent authority.

(2) The transporter must ensure that for each vehicle used for the transport of animals a register is kept containing the following information at least which shall be retained for a minimum period of three years -

(i) places and dates of pickup, and the name or business name and address of the holding of assembly centre where the animals are picked up;

(ii) places and dates of delivery, and the name or business name and address of the consignee(s);

(iii) species and number of animals carried;

(iv) date and place of disinfection;

(v) details of accompanying documentation, number, (serial number etc).

(3) Transporters shall ensure that the consignment or animals do not at any time, between leaving the holdings or the assembly centre of origin and arriving at their destination, come into contact with animals of a lower health status.

(4) Transporters shall give a written undertaking stating in particular that -

(i) all the measures necessary to comply with these regulations shall be taken and in particular the provisions laid down in this regulation and relating to the appropriate documentation that must accompany the animals,

(ii) the transport of animals is entrusted to staff who possess the necessary ability, professional competence and knowledge.

(5) In the event of failure to comply with the provisions of this regulation the requirements of Article 18 of European Union Council Directive 91/628/EEC shall apply *mutatis mutandis* relating to animal health.

#### **Approval number for dealers**

**13.** (1) In the territory of Malta all dealers are registered, approved and issued with an approval number by the competent authority and that they comply with the following conditions at least -

(a) they must deal only in animals that are identified and come from herds that are officially free of tuberculosis, brucellosis and leukosis or slaughter animals meeting the conditions set out in these regulations and in particular rule 6 (3). To this end, the dealer shall ensure that the animals are properly identified and are accompanied by health documents as appropriate for the species involved.

However, the competent authority may authorise the marketing of identified animals originating from the territory of Malta which do not fulfil the conditions laid down in this paragraph, in so far as they are brought direct to a slaughterhouse in the territory of Malta without passing through their facilities, for slaughter as soon as possible in order to prevent the spreading of diseases. The necessary provisions should be taken to ensure that such animals, when they reach the abattoir, cannot come into contact with other animals and that they are slaughtered apart from other animals;

(b) the dealer shall be required, either on the basis of the document accompanying the animals, or on the basis of identification numbers or

marks on the animals, to keep a record or data base and to store the following data for at least three years -

(i) the name of the owner, origin, date of purchase, categories, number and identification of bovine animals or registration number of the holding of origin or of the herd of origin of pigs purchased,

(ii) the registration number of the transporter and, or the licence number of the lorry delivering and collecting animals,

(iii) the name and address of the purchaser and the destination of the animal,

(iv) copies of route plans and, or serial number of health certificates as applicable;

(c) when the dealer keeps animals on his premises he shall ensure that -

(i) specific training is given to the staff in charge of the animals in applying the requirements of these rules and in the care and welfare of the animals,

(ii) controls and tests if necessary on the animals are carried out regularly by the official veterinarian and that all necessary steps are taken to prevent the spread of disease.

(2) In the territory of Malta all premises used a by dealer in connection with his business shall be registered and issued with an approval number by the competent authority and that they comply with the following conditions at least -

(a) they must be under the control of an official veterinarian;

(b) they must be located in an area which is not subject to prohibition or restrictions in accordance with relevant European Community legislation or Maltese legislation;

(c) they must have -

(i) appropriate facilities of sufficient capacity and in particular inspection facilities and isolation facilities so that all animals can be isolated in the event of an outbreak of a contagious disease,

(ii) appropriate facilities for unloading and where necessary adequate housing of a suitable standard for the animals, for watering and feeding them, and for giving them any necessary treatment; these facilities must be easy to clean and disinfect,

- (iii) an appropriate reception area for litter and manure,
  - (iv) an appropriate system for collecting waste water;
- (d) be cleaned and disinfected before use, as required by the official veterinarian.

(3) The competent authority may suspend or withdraw approval in the event of failure to comply with this rule or other appropriate provisions of these regulations or other regulations in respect of health restrictions. Approval may be restored when the Veterinary Services is satisfied that the dealer is in full compliance with all the appropriate provisions of these rules.

(4) The competent authority must carry out regular inspections in to ascertain that the requirements of this rule are fulfilled.

#### **Surveillance network system**

**14.** (1) The competent authority may carry out a system of surveillance network.

(a) The surveillance network system must comprise at least the following elements -

- (i) the herds;
- (ii) the owner or any other natural or legal person responsible for the holding;
- (iii) the approved veterinarian or the official veterinarian responsible for the holding;
- (iv) the competent authority of Malta;
- (v) the official veterinary diagnostic or any other laboratory approved by the competent authority;
- (vi) a computer database:

Provided that official veterinarians for the slaughtering establishments and approved assembly centres will be associated with the network system.

(2) The main objectives of the surveillance network system are to make the official classification of holdings, to maintain such classification by regular inspection, to collect epidemiological data and to carry out disease monitoring so as to ensure compliance with all the provisions of these regulations and other regulations in respect of

health restrictions:

Provided that this surveillance network system shall be mandatory on all holdings operating such a system in the territory of Malta. However, the competent authority may authorize the establishment of such a network on part of the territory made up of one or several adjacent regions. Where this derogation is accorded, animal movements to that part of the territory from other regions which are not part of the network system shall be subject to the provisions of these rules:

Further provided the competent authority shall lay down the obligations and rights incumbent upon the approved veterinarians, the persons responsible for the holdings or their owners and any other participants in the system including the persons responsible for issuing health certificates.

(3) The competent authority shall ensure that the obligations referred to in sub-rule at least include the following-

(a) Every owner of or person responsible for a holding must-

(i) secure, by contract or legal instrument, the services of a veterinarian approved by the competent authority;

(ii) immediately call in the approved veterinarian for the holding when he suspects the occurrence of an infectious disease or of any notifiable disease;

(iii) notify the approved veterinarian of all arrivals of animals on his holding;

(iv) isolate the animals before introducing them into his holding to enable the approved veterinarian to check, where appropriate by means of the required tests, whether the holding's status may be maintained.

(b) The approved veterinarians shall be under the control of the competent authority and must comply with the following requirements-

(i) meet the conditions for pursuing the veterinary profession;

(ii) have no financial interest or family links with the owner of or person responsible for the holding;

(iii) possess particular knowledge in the field of animal health as it applies to animals of the species concerned. This means that they must-

- regularly update their knowledge, especially as regards the relevant health regulations;
- meet the requirements laid down by the competent authority to ensure the proper functioning of the network;
- provide the owner of or person responsible for the holding with information and assistance in order that all steps are taken to ensure that the holding's status is maintained, particularly on the basis of programmes agreed with the competent authority,
- ensure compliance with the requirements concerning-
  - (i) the identification and health certification of the animals of the herd, the animals introduced and those traded;
  - (ii) compulsory reporting of infectious animal diseases and any other risk factor for animal health or welfare, and for human health;
  - (iii) establishing as far as possible the cause of death of animals and where they are to be consigned;
  - (iv) the hygiene conditions of the herd and of the livestock production units:

Provided that if the proper functioning of the system so requires, each Member State may limit the veterinarians' responsibility to a specific number of holdings or to a specific geographical area:

Provided further that the competent authority shall draw up lists of approved Veterinarians and of the approved holdings participating in the network. If the competent authority finds that a participant in the network no longer fulfils the conditions set out above, it shall suspend or withdraw approval, without prejudice to any penalties that may be applied.

(c) The computer database must contain at least the following information-

(i) For each animal-

- identification code;

- date of birth;
- sex;
- breed or colour of coat;
- identification code of the mother or, in the case of an animal imported from a third country, the identification number given following inspection under European Union Council Directive 92/102/EEC and corresponding to the identification number of origin;
- identification number of the holding where born;
- identification numbers of all holdings where the animal has been kept and the dates of each change of holding;
- date of death or slaughter.

(ii) For each holding-

- an identification number consisting of not more than 12 figures (apart from the country code);
- name and address of the holder.

(iii) The database must be able to supply the following particulars at any time-

- the identification number of all animals of the bovine species present on a holding, or in the case of groups of animals of the porcine species, the registration number of the holding of origin or herd of origin and the number of the health certificate where applicable,
- a list of all changes of holding for each animal of the bovine species starting from the holding of birth, or the holding of importation in the case of animals imported from third countries; and for groups of pigs the registration number of the last holding or last herd and for imported animals from third countries the holding of importation:

Provided that these particulars will be held on the database until three consecutive years have elapsed since the death of the bovine animal or until three consecutive years have

elapsed since the record was made in the case of records for pigs. However, only points 2, 3 and 4 shall be applicable to porcine animals.

(iv) In order to ensure the operation of the national computer databases concerning porcine animals, appropriate rules of application, including the information that the national databases must contain, shall be adopted in accordance with the procedure laid down in Article 17 of the European Union Council Directive 64/432/EEC.

(4) All participants in the surveillance network other than those provided for in 3A and B shall be accountable to the competent authority. The competent authority in each Member State shall be responsible for setting up the network and shall carry out regular checks to ensure that it operates properly.

(5) Member States which introduce a system of surveillance networks as outlined in regulation 14 (3) (c), operational for a period of at least 12 months, shall apply to the Commission to have it approved under the procedure provided for in regulation 17:

Provided that for this purpose the European Commission shall examine documentation submitted by Member States.

(6) When the competent authority has implemented a through out its jurisdiction a recognized surveillance network system as laid down in this regulation shall be authorized not to apply the provision referred to in Article 3 (2) (a), second indent to animal movements referred to by this regulation within their own territory.

(7) The financing of the surveillance network system will be covered within the framework of the revision of Annex B to European Union Council Directive 85/73/EEC in accordance with the provisions laid down in Article 8 of European Union Council Directive 96/43/EC.

### **Penalties**

**15.** (1) Any infringement of these regulations whether by a natural or a legal person shall be penalised by the appropriate specific measures laid down in the Maltese legislation.

(2) If it is confirmed that at the arrival in Malta the provisions of these regulations are not or have not been complied with, the competent authority shall take all appropriate measures to safeguard animal health and to prevent the spread of disease. Depending on the circumstances, such action by the competent authority may consist of taking the measures required to -

(a) arrange for the journey to be completed or the animals returned to their place of departure by the most direct route, providing this course of action would not further jeopardise the health or welfare of the animals;

(b) arrange for the animals to be held in suitable accommodation with appropriate care in the event of interruption of the journey;

(c) arrange for the slaughter of the animals. The destination and use of such animals after slaughter shall be regulated -

(i) in accordance with the provisions of European Union Council Directive 64/433/EEC on fresh meat; or

(ii) in accordance with the provisions of the European Union Council Directive 90/667/EEC where the health status of the animals cannot be established or where they are liable to be a risk to animal health or public health. However, where the provisions of European Union Council Directive 90/667/EEC should apply, a regularization period may be accorded to the owner or his agent before that final possibility is invoked. In that case, the provisions of sub-rule (3) shall apply;

(3) The Competent Authority shall immediately notify the competent authority of the Member State of origin on the establishment of any infringement of these regulations. In accordance with the provisions established by European Union Council Directive 89/608/EEC, the competent authority shall provide mutual assistance to one another Member State in the application of these regulations in order to ensure, in particular, compliance with the provisions laid down in this regulation.

(4) This regulation shall not affect other national rules applicable to penal sanctions.

#### **Interim period between computer database and network system**

**16.** (1) Pending the introduction in Malta of an approved surveillance network system a computer database complying with the provisions laid down in regulation 14 shall be fully operational for bovine animals on the 31 December 2002 at the latest:

Provided that with regards to porcine animals, there shall be an entry in the database for each separate movement of porcine animals. The entry shall comprise at least the following -

(i) the number of porcine animals being moved,

(ii) the identification number of the holding or herd of departure,

(iii) the identification number of the holding or herd of arrival, and

(iv) the departure and arrivals dates.

The recording of all movements of porcine animals shall be fully operational on the 31 December 2003 at the latest.

**Contract to be established between the Maltese competent authority and listed laboratories or Institutes**

17. A contract may be established between the competent authority of Malta and the laboratories or Institutes listed in the Annex B, Annex C, Annex D, chapter II of European Union Council Directive 64/432/EEC and Annex III of European Union Commission Decision 2001/618/EC, for the purpose of the tasks indicated in the relevant Annexes.

**Checks at origin and at destination**

18. The competent authority shall ensure that the rules laid down in the European Union Council Directive 90/425/EEC shall apply in particular to checks at origin, to the organization of, and follow-up to, the checks to be carried out by the country of destination, and to the safeguard measures to be implemented.

**SCHEDULE A, B, C, D, E and F to the European council Directive 64/432/EEC**  
**SCHEDULE A**

**I. Officially tuberculosis-free bovine herd**

For the purposes of this section “bovine animals” means all bovine animals with the exception of animals taking part in cultural or sporting events.

1. A bovine herd is officially tuberculosis-free if -

- (a) all the animals are free from clinical signs of tuberculosis;
- (b) all the bovine animals over six weeks old have reacted negatively to at least two official intra-dermal tuberculin tests carried out in accordance with Schedule B, the first six months after the elimination of any infection from the herd and the second six months later or, where the herd has been assembled solely from animals that originate in officially tuberculosis-free herds, the first test shall be carried out at least 60 days after assembly and the second shall not be required;
- (c) following the completion of the first test referred to in (b), no bovine animal over six weeks old has been introduced into the herd unless it has reacted negatively to an intra-dermal tuberculin test performed and assessed according to Schedule B and carried out either in the 30 days prior to, or the 30 days after the date of its introduction into the herd; in the latter case the animal(s) must be isolated physically from the other animals of the herd in a way to avoid any direct or indirect contact with the other animals until proven negative.

However, the competent authority may not require this test to be carried out for movements of animals on its own territory if the animal is from an officially tuberculosis-free herd, except in a Member State where, on 1 January 1998 and until the status of officially tuberculosis-free region is obtained, the competent authority required such tests to be carried out for animals moving between herds participating in a network system as referred to in regulation 14.

2. A bovine herd will retain officially tuberculosis-free status if -

- (a) the conditions detailed in 1(a) and (c) continue to apply;
- (b) all animals entering the holding come from herds of officially tuberculosis-free status;
- (c) all animals on the holding, with the exception of calves under six weeks old which were born in the holding, are subjected to routine tuberculin testing in accordance with Schedule B at yearly intervals.

However, the competent authority of a Member State may, for the Member State or part of the Member State where all the bovine herds are subject to an official programme to combat tuberculosis, alter the frequency of the routine tests as follows -

if the average — determined at 31 December of each year — of the annual percentages of bovine herds confirmed as infected with tuberculosis is not more than 1 % of all herds within the defined area during the two most recent annual supervisory periods, the interval between routine herd tests may be increased to two years and male animals for fattening within an isolated epidemiological unit may be exempted from tuberculin testing provided that they come from officially tuberculosis-free herds and that the competent authority guarantees that the males for fattening will not be used for breeding and will go direct for slaughter,

if the average — determined at 31 December of each year — of the annual percentages of bovine herds confirmed as infected with tuberculosis is not more than 0,2 % of all herds within the defined area during the two most recent biennial supervisory periods, the interval between routine tests may be increased to three years and/or the age at which animals have to undergo these tests may be increased to 24 months,

if the average — determined at 31 December of each year — of the annual percentages of bovine herds confirmed as infected with tuberculosis is not more than 0,1 % of all herds within the defined area during the two most recent supervisory triennial periods, the interval between routine tests may be increased to four years, or, providing the following conditions are met, the competent authority may dispense with tuberculin testing of the herds -

(1) before the introduction into the herd all the bovine animals are subjected to an intra-dermal tuberculin test with negative results;

or

(2) all bovine animals slaughtered are examined for lesions of tuberculosis and any such lesions are submitted to a histopathological and bacteriological examination for evidence of tuberculosis.

The competent authority may also, in respect of the Member State or a part thereof, increase the frequency of tuberculin testing if the level of the disease has increased.

3A. The officially tuberculosis-free status of a herd is to be suspended if -

(a) the conditions detailed in paragraph 2 are no longer fulfilled; or

(b) one or more animals are deemed to have given a positive reaction to a tuberculin test, or a case of tuberculosis is suspected at post-mortem examination.

When an animal is considered to be a positive reactor it will be removed from the herd and slaughtered. Appropriate post-mortem, laboratory and epidemiological examinations shall be carried out on the positive reactor or the carcase of the suspect animal. The status of the herd will remain suspended until such time as all laboratory examinations have been completed. If the presence of tuberculosis is not confirmed, the suspension of the officially tuberculosis-free status may be lifted following a test of all animals over six weeks of age with negative results at least 42 days after the removal of the reactor animal(s);

or

(c) the herd contains animals of unresolved status as described in Schedule B. In this case, the status of the herd is to remain suspended until the animals' status has been clarified. Such animals must be isolated from the other animals of the herd until their status has been clarified, either by a further test after 42 days or by post-mortem and laboratory examination;

(d) however, by way of derogation from the requirements of paragraph (c), in a Member State where the competent authority carries out routine herd testing using the comparative tuberculin test described in Schedule B, and in the case of a herd where no confirmed reactor animals have been disclosed for at least three years, the competent authority may decide not to restrict the movement of other animals in the herd, provided that the status of any inconclusive reactors is resolved by a further test after 42 days and that no animals from the holding are allowed to enter into intra-Community trade until the status of any inconclusive reactors has been resolved. If at this further test any animal either gives a positive reaction or continues to give an inconclusive reaction, then the

conditions of paragraph (b) apply. If the presence of disease is subsequently confirmed, all animals leaving the holding since the time of the last clear herd test must be traced and tested.

3B. The officially tuberculosis-free status of the herd is to be withdrawn if the presence of tuberculosis is confirmed by the isolation of *M. bovis* on laboratory examination.

The competent authority may withdraw status if -

- (a) the conditions detailed in point 2 are no longer fulfilled, or
- (b) classical lesions of tuberculosis are seen at post-mortem examination, or
- (c) an epidemiological enquiry establishes the likelihood of infection,
- (d) or for any other reasons considered necessary for the purpose of controlling bovine tuberculosis.

Tracing and checking is to be undertaken by the competent authority of any herd considered to be epidemiologically related. The officially tuberculosis-free status of a herd is to remain withdrawn until cleansing and disinfection of the premises and utensils has been completed and all animals over six weeks of age have reacted negatively to at least two consecutive tuberculin tests, the first no less than 60 days and the second no less than four months and no more than 12 months after the removal of the last positive reactor.

4. On the basis of information supplied in accordance with regulation 8, a Member State or part of a Member State may be declared officially tuberculosis-free according to the procedure laid down in Article 17 of the European Union Council Directive 64/432/EEC if it meets the following conditions -

- (a) the percentage of bovine herds confirmed as infected with tuberculosis has not exceeded 0,1 % per year of all herds for six consecutive years and at least 99,9 % of herds have achieved officially tuberculosis-free status each year for six consecutive years, the calculation of this latter percentage to take place on 31 December each calendar year;
- (b) an identification system making it possible to identify the herds of origin and transit for each bovine animal is in existence in accordance with European Union Regulation (EC) No. 1760/2000;
- (c) all bovine animals slaughtered are subjected to an official post-mortem examination;
- (d) the procedures for suspension and withdrawal of officially tuberculosis-free status are complied with.

5. The Member State or part of a Member State will retain officially tuberculosis-free status if the conditions 4(a) to (d) continue to be met. However, if there is evidence of a significant change in the situation as regards tuberculosis in a Member State or part of a Member State which has been recognised as officially tuberculosis-free, the European Commission may, in accordance with the procedure laid down in Article 17 of the European Union Council Directive 64/432/EEC, take a Decision suspending or revoking the status until the requirements of the Decision have been fulfilled.

## **II. Officially brucellosis-free and brucellosis-free bovine herds**

For the purposes of this section “bovine animals” means all bovine animals with the exception of males for fattening provided that they come from officially brucellosis-free herds and that the competent authority guarantees that the males for fattening will not be used for breeding and will go direct for slaughter.

1. A bovine herd is officially brucellosis-free if -

(a) it contains no bovine animals which have been vaccinated against brucellosis, except females which have been vaccinated at least three years previously;

(b) all the bovine animals have been free from clinical signs of brucellosis for at least six months;

(c) all the bovine animals over 12 months old have been subjected to one of the following test regimes with negative results in accordance with Schedule C -

(i) two serological tests specified in paragraph 10 at an interval of more than three months and less than 12 months;

(ii) three tests on milk samples at three-monthly intervals followed at least six weeks later by a serological test specified in paragraph 10;

(d) any bovine animal entering the herd comes from a herd of officially brucellosis-free status and, in the case of bovine animals over 12 months old, has shown a brucella titre of less than 30 IU of agglutination per ml when given a serum agglutination test in accordance with Schedule C or has reacted negatively to any other test approved in accordance with the procedure at Article 17 during the 30 days prior to or the 30 days after the date of its introduction into the herd: in the latter case, the animal(s) must be isolated physically from the other animals of the herd in such a way as to avoid direct or indirect contact with the other animals until proven negative.

2. A bovine herd will retain officially brucellosis-free status if -

(a) one of the following test regimes is carried out annually with negative results in accordance with Schedule C -

(i) three milk ring tests carried out at intervals of at least three months;

(ii) three milk ELISAs carried out at intervals of at least three months;

(iii) two milk ring tests carried out at an interval of at least three months followed at least six weeks later by a serological test referred to in paragraph 10;

(iv) two milk ELISAs carried out at an interval of at least three months followed at least six weeks later by a serological test referred to in paragraph 10;

(v) two serological tests carried out at an interval of at least three months and not more than 12 months.

However, the competent authority of a Member State may, for the Member State or part of the Member State which is not officially brucellosis-free but where all the bovine herds are subject to an official programme to combat brucellosis, alter the frequency of the routine tests as follows -

— where not more than 1 % of bovine herds are infected, it may be sufficient to carry out each year two milk ring tests or two milk ELISAs at an interval of at least three months, or one serological test,

— where at least 99,8 % of bovine herds have been recognised as officially brucellosis-free for at least four years, the interval between checks may be extended to two years if all animals over 12 months of age are tested, or testing may be restricted to animals over 24 months of age if herds continue to be tested each year. The checks must be carried out using one of the serological tests referred to in paragraph 10;

(b) all bovine animals entering the herd come from herds of officially brucellosis-free status and, in the case of bovine animals over 12 months old, have shown a brucella titre of less than 30 IU of agglutination per ml when given a serum agglutination test in accordance with Schedule C or have reacted negatively to any other test approved in accordance with the procedure at Article 17 during the 30 days prior to or the 30 days after the date of their introduction into the herd; in the latter case, the animal(s) must be isolated physically from the other animals of the herd in such a way as to avoid direct or indirect contact with the other animals until proven negative.

However, the test described in point (b) need not be required in Member States, or regions of Member States, where the percentage of bovine herds infected with brucellosis has not exceeded 0,2 % for at least two years and where the animal comes from an officially brucellosis-free bovine herd within that Member State or region and has not during transportation come into contact with bovine animals of lesser status;

(c) notwithstanding point (b), bovine animals from a brucellosis-free bovine herd may be introduced into an officially brucellosis-free herd if they are at least 18 months old and, if vaccinated against brucellosis, the vaccination was carried out more than a year previously.

Such animals must have shown, in the 30 days prior to introduction, a brucella titre lower than 30 IU of agglutination per ml and a negative result when given a complement fixation test, or other test approved under the procedure set out in Article 17 of the European Union Council Directive 64/432/EEC.

If, however, a female bovine animal from a brucellosis-free herd is introduced into an officially brucellosis-free herd, under the provisions of the above paragraph, that herd shall be considered to be brucellosis-free for two years from the date on which the last vaccinated animal was introduced.

3A. The officially brucellosis-free status of a herd is to be suspended if -

(a) the conditions detailed in paragraphs 1 and 2 are no longer fulfilled; or

(b) as a result of laboratory tests or on clinical grounds one or more bovine animals is suspected of having brucellosis and the suspect animals have been slaughtered or isolated in a way to avoid any direct or indirect contact with the other animals.

Where the animal has been slaughtered and is no longer available for testing, the suspension may be lifted if two serum agglutination tests, carried out in accordance with Schedule C on all bovine animals in the herd over 12 months old, show a titre lower than 30 IU of agglutination per ml. The first test shall be carried out at least 30 days after the removal of the animal and the second at least 60 days later.

Where the animal has been isolated from the animals in the herd, it may be reintroduced into the herd and the status of the herd may be restored following -

(a) a serum agglutination test which has shown a titre lower than 30 IU of agglutination per ml and has given a negative result to a complement fixation test, or

(b) a negative result to any other combination of tests approved for that purpose under the procedure set out in Article 17 of the European Union Council Directive 64/432/EEC.

3B. The officially brucellosis-free status of the herd is to be withdrawn if, as a result of laboratory tests or epidemiological investigations, brucella infection has been confirmed in the herd.

The status of the herd is not to be restored until either all bovine animals present in the herd at the time of the outbreak have been slaughtered, or the herd has been subject to check testing and all animals

over 12 months of age have given negative results to two consecutive tests at 60-day intervals, the first being carried out not less than 30 days after removal of the positive animal(s).

In the case of bovine animals which were pregnant at the time of the outbreak, the final check must be carried out at least 21 days after the last animal pregnant at the time of the outbreak has calved.

4. A bovine herd is brucellosis-free if it complies with the conditions in 1(b) and (c) and when vaccination has been carried out as follows -

(i) female bovine animals have been vaccinated -

— before the age of six months old with live strain 19 vaccine, or

— before the age of 15 months old with killed 45/20 adjuvant vaccine which has been officially inspected and approved, or

— with other vaccines approved under the procedure laid down in Article 17 of the European Union Council Directive 64/432/EEC;

(ii) bovine animals under 30 months old which have been vaccinated with live strain 19 vaccine may give a serum agglutination test result greater than 30 IU but less than 80 IU of agglutination per millilitre provided that, on the complement fixation test, they give a result less than 30 EEC units in the case of females vaccinated less than 12 months previously or less than 20 EEC units in all other cases.

5. A bovine herd will retain brucellosis-free status if -

(i) it is subject to one of the testing regimes listed in 2(a);

(ii) bovine animals entering the herd comply with the requirements of 2(b); or

— come from herds of brucellosis-free status, and in the case of bovine animals over 12 months old, have shown, in the 30 days prior to or in isolation after introduction into the herd, less than 30 IU of agglutination per ml when given a serum agglutination test and a negative result to a complement fixation test in accordance with Schedule C, or

— come from herds of brucellosis-free status, are under 30 months old and have been vaccinated with live strain 19 vaccine if they give a serum agglutination test result greater than 30 IU but less than 80 IU of agglutination per millilitre provided that, on the complement fixation test, they give a result less than 30 EEC units in the case of females vaccinated less than 12 months previously or less than 20 EEC units in all other cases.

6A. The brucellosis-free status of a herd is to be suspended if -

(a) the conditions detailed in paragraphs 4 and 5 have not been complied with; or

(b) as a result of laboratory tests or on clinical grounds one or more bovine animals over 30 months old is suspected of having brucellosis and the animal(s) under suspicion have been slaughtered, or isolated in a way to avoid any direct or indirect contact with other animals.

Where the animal has been isolated, it may be reintroduced into the herd and the status of the herd may be restored, if it subsequently shows a serum agglutination titre lower than 30 IU of agglutination per ml and has given a negative result to a complement fixation test, or other test approved under the procedure set out in Article 17 of the European Union Council Directive 64/432/EEC.

Where the animals have been slaughtered and are no longer available for testing, the suspension may be lifted if two serum agglutination tests, carried out in accordance with Schedule C on all bovine animals in the holding over 12 months old, show a titre lower than 30 IU of agglutination per ml. The first test is to be carried out at least 30 days after the removal of the animal and the second at least 60 days later.

If the animals to be tested in the previous two subparagraphs are under 30 months old and have been vaccinated with live strain 19 vaccine they may be considered to be negative if they give a serum agglutination test result greater than 30 IU but less than 80 IU of agglutination per millilitre provided that, on the complement fixation test, they give a result less than 30 EEC units in the case of females vaccinated less than 12 months previously or less than 20 EEC units in all other cases.

- 6B. The brucellosis-free status of the herd is to be withdrawn if, as a result of laboratory tests of epidemiological investigations, brucella infection has been confirmed in a herd. The status of the herd is not to be restored until either all the bovine animals present in the herd at the time of the outbreak have been slaughtered or the herd has been subject to check testing and all unvaccinated animals over 12 months of age have given negative results to two consecutive tests at 60 day intervals, the first being carried out not less than 30 days after removal of the positive animal(s).

If all the animals to be tested referred to in the preceding paragraph are less than 30 months old and have been vaccinated with live strain 19 vaccine, they may be considered negative if they show a brucella titre of more than 30 IU but less than 80 IU of agglutination per ml, provided that in the complement fixation test they show a titre of less than 30 EEC units in the case of females vaccinated less than 12 months previously or a titre of less than 20 EEC units in all other cases.

In the case of bovine animals which were pregnant at the time of the outbreak, the final check must have been carried out at least 21 days after the last animal pregnant at the time of the outbreak has calved.

7. A Member State or a region of a Member State may be declared officially brucellosis-free according to the procedure laid down in Article 17 of the European Union Council Directive 64/432/EEC if it meets the following conditions -

(a) no case of abortion due to brucella infection and no isolation of *B. abortus* has been recorded for at least three years and at least 99,8 % of herds have achieved officially brucellosis-free status each year for five consecutive years, the calculation of this percentage to take place on 31 December each calendar year. However, where the competent authority adopts a policy of whole herd slaughter, isolated incidents shown by epidemiological enquiry to be due to the introduction of animals from outside the Member State or part of the Member State and herds whose officially brucellosis-free status has been suspended or withdrawn for reasons other than suspicion of disease, is to be disregarded for the purpose of the above calculation provided that the central competent authority of the Member State concerned by these incidents makes an annual record and forwards them to the European Commission in accordance with regulation 8(2), and

(b) an identification system making it possible to identify the herds of origin and transit for each bovine animal is in existence in accordance with European Union Regulation (EC) No. 820/97, and

(c) notification of cases of abortion is mandatory and they are investigated by the competent authority.

8. Subject to paragraph 9, a Member State or a region of a Member State declared officially brucellosis free is to retain this status if -

- (a) the conditions imposed by paragraph 7(a) and (b) are still fulfilled and notification of cases of abortion suspected of being due to brucellosis is mandatory and are investigated by the competent authority;
- (b) every year for the first five years after attaining status, all bovine animals over 24 months of age in not less than 20 % of herds have been tested and have reacted negatively to a serological test carried out in accordance with Schedule C or, in the case of dairy herds, by examination of milk samples in accordance with Schedule C;
- (c) every bovine animal suspected of being infected with brucellosis is notified to the competent authority and undergoes official epidemiological investigation for brucellosis comprising at least two serological blood tests, including the complement fixation test, and a microbiological examination of appropriate samples;
- (d) during the period of suspicion, which is to continue until negative results have been obtained from the tests provided for in (c), the officially brucellosis-free status of the herd of origin or transit of the suspected bovine animal and of the herds linked epidemiologically to it is to be suspended;
- (e) in the event of an outbreak of brucellosis that has spread, all bovine animals have been slaughtered. Animals of the remaining susceptible species will undergo appropriate tests and premises and equipment will be cleaned and disinfected.
9. A Member State or a region of a Member State declared officially brucellosis-free is to report the occurrence of all cases of brucellosis to the European Commission. If there is evidence of a significant change in the situation as regards brucellosis in a Member State or part of a Member State which has been recognised as officially brucellosis-free, the European Commission may according to the procedure laid down in Article 17 of the European Union Council Directive 64/432/EEC propose that the status be suspended or revoked until the requirements of the Decision have been fulfilled.
10. For the purposes of section II, a serological test means either a serum agglutination test, buffered brucella antigen test, complement fixation test, plasma agglutination test, plasma ring test, micro-agglutination test or individual blood ELISA, as described in Schedule C. Any other diagnostic test approved under the procedure laid down in Article 17 of the European Union Council Directive 64/432/EEC and described in Schedule C will also be accepted for the purposes of section II. A milk test means a milk ring test or a milk ELISA in accordance with Schedule C.

## SCHEDULE B

### TUBERCULOSIS

#### 1. IDENTIFICATION OF THE AGENT

The presence of *Mycobacterium bovis* (*M. bovis*), agent of bovine tuberculosis, in clinical and post-mortem specimens may be demonstrated by examination of stained smears or immunoperoxidase techniques and confirmed by cultivation of the organism on primary isolation medium.

Pathological material for the confirmation of *M. bovis* should be taken from abnormal lymph nodes and parenchymatous organs such as lungs, liver, spleen, etc. In the cases where the animal does not present pathological lesions, samples from the retropharyngeal, bronchial, mediastinal, supramammary, mandibular and some mesenteric lymph nodes and liver should be collected for examination and culture.

Identification of isolates may be usually carried out by determining cultural and biochemical properties. The polymerase chain reaction (PCR) may also be employed for the detection of the

*M. tuberculosis* complex. DNA analysis techniques may prove to be faster and more reliable than biochemical methods for the differentiation of *M. bovis* from other members of the *M. tuberculosis* complex. Genetic fingerprinting allows distinguishing between different strains of *M. bovis* and will enable patterns of origin, transmission and spread of *M. bovis* to be described.

The techniques and media used, their standardisation and the interpretation of results must conform to that specified in the OIE Manual of Standards for Diagnostic Tests and Vaccines, Fourth Edition, 2000, Chapter 2.3.3 (bovine tuberculosis).

## 2. THE TUBERCULIN SKIN TEST

Tuberculin PPD (Purified Protein Derivatives) that fulfil the standards laid down in paragraph 2.1 shall be used for carrying out official tuberculin skin test following the procedures referred to in paragraph 2.2.

### 2.1. Standards for tuberculin (bovine and avian)

#### 2.1.1. *Definition*

Tuberculin purified protein derivative (tuberculin PPD, bovine or avian) is a preparation obtained from the heat-treated products of growth and lysis of *Mycobacterium bovis* or *Mycobacterium avium* (as appropriate) capable of revealing a delayed hypersensitivity in an animal sensitised to microorganisms of the same species.

#### 2.1.2. *Production*

It is obtained from the water-soluble fractions prepared by heating in free-flowing steam and subsequently filtering cultures of *M. bovis* or *M. avium* (as appropriate) grown in a liquid synthetic medium. The active fraction of the filtrate, consisting mainly of protein, is isolated by precipitation, washed and re-dissolved. An antimicrobial preservative that does not give rise to false positive reactions, such as phenol, may be added. The final sterile preparation, free from mycobacteria, is distributed aseptically into sterile tamper-proof glass containers which are then closed so as to prevent contamination. The preparation may be freeze-dried.

#### 2.1.3. *Identification of the product*

Inject a range of graded doses intradermally at different sites into suitably sensitised albino guinea-pigs, each weighing not less than 250 g. After 24 h to 28 h, reactions appear in the form of oedematous swellings with erythema with or without necrosis at the points of injection. The size and severity of the reactions vary according to the dose. Unsensitised guinea-pigs show no reactions to similar injections.

#### 2.1.4. *Tests*

2.1.4.1. **pH:** The pH is 6.5 to 7.5.

2.1.4.2. **Phenol:** If the preparation to be examined contains phenol, its concentration is not more than 5 g/l.

2.1.4.3. **Sensitising effect:** Use a group of three guinea-pigs that have not been treated with any material which will interfere with the test. On 3 occasions at intervals of five days inject intradermally into each guinea-pig a dose of the preparation to be examined equivalent to 500 IU in 0,1 ml. 15 to 21 days after the third injection inject the same dose (500 IU) intradermally into these animals and into a control group of three guinea-pigs of the same mass and which have not previously received injections of tuberculin. 24 to 28 hours after the last injections, the reactions of the two groups are not significantly different.

2.1.4.4. **Toxicity:** Use two guinea-pigs, each weighing not less than 250 g and which have not previously been treated with any material which will interfere with the test. Inject subcutaneously

into each guinea-pig 0,5 ml of the preparation to be examined. Observe the animals for seven days. No abnormal effects occur during the observation period.

2.1.4.5. **Sterility**: It complies with the test for sterility prescribed in the monograph on Vaccines for veterinary use 4th Edition 2002 of the European Pharmacopoeia.

2.1.5. **Potency**

The potency of tuberculin purified protein derivative (bovine and avian) is determined by comparing the reactions produced in sensitised guinea-pigs by the intradermal injection of a series of dilutions of the preparation to be examined with those produced by known concentrations of a reference preparation of tuberculin (bovine or avian, as appropriate) purified protein derivative calibrated in international Units.

To test the potency, sensitise not fewer than nine albino guinea-pigs, each weighing 400 g to 600 g, by the deep intramuscular injection of 0,0001 mg of wet mass of living *M. bovis* of strain AN5 suspended in 0.5 ml of a 9 g/l solution of sodium chloride R for bovine tuberculin, or a suitable dose of inactivated or live *M. avium* for avian tuberculin. Not less than four weeks after the sensitisation of the guinea-pigs, shave their flanks to provide space for not more than four injection sites on each side. Prepare dilutions of the preparation to be examined and of the reference preparation using isotonic phosphate-buffered saline (pH 6,5-7,5) containing 0,005 g/l of polysorbate 80 R. Use not fewer than three doses of the reference preparation and not fewer than three doses of the preparation to be examined. Choose the doses such that the lesions produced have a diameter of not less than 8 mm and not more than 25 mm. Allocate the dilutions randomly to the sites using a Latin square design. Inject each dose intradermally in a constant volume of 0,1 ml or 0,2 ml. Measure the diameters of the lesions after 24 to 28 hours and calculate the result of the test using the usual statistical methods and assuming that the diameters of the lesions are directly proportional to the logarithm of the concentration of the tuberculins.

The test is not valid unless the fiducial limits of error ( $P = 0,95$ ) are not less than 50 % and not more than 200 % of the estimated potency. The estimated potency is not less than 66 % and not more than 150 % of the stated potency for bovine tuberculin. The estimated potency is not less than 75 % and not more than 133 % of the stated potency for avian tuberculin. The stated potency is not less than 20 000 IU/ml for both tuberculins (bovine and avian).

2.1.6. **Storage**

Store protected from light, at a temperature of  $5 \pm 3$  °C.

2.1.7. **Labelling**

The label states -

- the potency in International Units per millilitre,
- the name and quantity of any added substance,
- for freeze-dried preparations:
  - (a) the name and volume of the reconstituting liquid to be added,
  - (b) that the product should be used immediately after reconstitution.

2.2. **Test procedures**

2.2.1. The following shall be recognised as official intra- dermal tuberculin tests -

- the single intradermal test: this test requires a single injection of bovine tuberculin,

- the intradermal comparative test: this test requires one injection of bovine tuberculin and one injection of avian tuberculin given simultaneously.

2.2.2. The dose of tuberculin injected shall be -

- not less than 2 000 IU of bovine tuberculin,

- not less than 2 000 IU of avian tuberculin.

2.2.3. The volume of each injection dose shall not exceed 0,2 ml.

2.2.4. Tuberculin tests shall be carried out by injecting tuberculin(s) into the skin of the neck. The injection sites shall be situated at the back of the anterior and middle thirds of the neck. When both avian and bovine tuberculins are injected in the same animal, the site for injection of avian tuberculins shall be about 10 cm from the crest of the neck and the site for the injection of bovine tuberculin about 12,5 cm lower on a line roughly parallel with the line of the shoulder or on different sides of the neck; in young animals in which there is not room to separate the sites sufficiently on one side of the neck, one injection shall be made on each side of the neck at identical sites in the centre of the middle third of the neck.

2.2.5. The technique of tuberculin testing and interpretation of reactions shall be as follows -

2.2.5.1. *Technique -*

Injection sites shall be clipped and cleansed. A fold of skin within each clipped area shall be taken between the forefinger and thumb and measured with callipers and recorded. The dose of tuberculin shall then be injected by a method that ensures that the tuberculin is delivered intradermically. A short sterile needle, bevel edge outwards, with graduated syringe charged with tuberculin, inserted obliquely into the deeper layers of the skin may be used. A correct injection shall be confirmed by palpating a small pea-like swelling at each site of injection. The skin-fold thickness of each injection site shall be remeasured 72 hours ( $\pm$  4 hours) after injection and recorded.

2.2.5.2. *Interpretation of reactions*

The interpretation of reactions shall be based on clinical observations and the recorded increase(s) in skin-fold thickness at the sites of injection 72 hours after injection of tuberculin(s).

(a) Negative reaction: if only limited swelling is observed, with an increase of not more than 2 mm in the thickness of the fold of skin without clinical signs such as diffuse or extensive oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts in that region or of the lymph nodes.

(b) Inconclusive reaction: if no clinical signs such as mentioned in (a) are observed and if the increase in skin-fold thickness is more than 2 mm and less than 4 mm.

(c) Positive reaction: if clinical signs such as mentioned in (a) are observed or there is an increase of 4 mm or more in the thickness of the fold of skin at the injection site.

2.2.5.3. The interpretation of official intradermal tuberculin tests shall be as follows -

2.2.5.3.1. Single intradermal test-

(a) positive: a positive bovine reaction as defined in paragraph 2.2.5.2(c);

(b) inconclusive: an inconclusive reaction as defined in paragraph 2.2.5.2(b);

(c) negative: a negative bovine reaction as defined in paragraph 2.2.5.2(a).

Animals inconclusive to the single intradermal test shall be subjected to another test after a minimum of 42 days.

Animals which are not negative to this second test shall be deemed to be positive to the test.

Animals positive to the single intradermal test may be subjected to an intradermal comparative test if false positive reaction or interference reaction is suspected.

2.2.5.3.2. Intradermal comparative test for the establishment and maintenance of officially tuberculosis-free herd status -

(a) positive: a positive bovine reaction which is more than 4 mm greater than the avian reaction, or the presence of clinical signs;

(b) inconclusive: a positive or inconclusive bovine reaction which is from 1 to 4 mm greater than the avian reaction, and the absence of clinical signs;

(c) negative: a negative bovine reaction, or a positive or inconclusive bovine reaction but which is equal to or less than a positive or inconclusive avian reaction and the absence of clinical signs in both cases.

Animals inconclusive to the intradermal comparative test shall be subjected to another test after a minimum of 42 days. Animals, which are not negative to this second test, shall be deemed to be positive to the test.

2.2.5.3.3. Officially tuberculosis-free herd status may be suspended and animals from the herd shall not be allowed to enter intra-Community trade until such time as the status of the following animals is resolved -

(a) animals which have been deemed to be inconclusive to the single intradermal tuberculin test;

(b) animals which have been deemed to be positive to the single intradermal tuberculin test but are awaiting retest with an intradermal comparative test;

(c) animals which have been deemed to be inconclusive to the intradermal comparative test.

2.2.5.3.4. Where animals are required by European Community legislation to be subjected to an intradermal test prior to movement, the test shall be interpreted so that no animal which shows an increase in skin-fold thickness greater than 2 mm or the presence of clinical signs is entered into Intra-Community trade.

2.2.5.3.5. To enable detection of the maximum number of infected and diseased animals in a herd or in a region, Member States may modify the criteria for the interpretation of the test in to achieve improved test sensitivity considering all inconclusive reactions referred in 2.2.5.3.1(b) and 2.2.5.3.2(b) as positive reactions.

3. SUPPLEMENTARY TESTING

To enable detection of the maximum number of infected and diseased animals in a herd or in a region, Member States may authorise the employ of the gamma-interferon assay referred in the OIE Manual of Standards for Diagnostic Tests and Vaccines, 4th Edition, 2000, Chapter 2.3.3. (bovine tuberculosis), in addition to the tuberculin test.

4. STATE INSTITUTES AND NATIONAL REFERENCE LABORATORIES

4.1. **Tasks and responsibilities**

The State Institutes and Reference Laboratories included in paragraph 3.2 shall be responsible for the official testing of tuberculins or reagents included in paragraph 2 and 3 in their respective States to ensure each of these tuberculins or reagents are adequate in relation to the standards above referred.

4.2. List of State institutes and national reference laboratories

1. Germany:

Paul-Ehrlich Institut (PEI), Bundesamt für Sera und Impfstoffe, D-23207 Langen; Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin - Bereich Jena - D-07743 Jena;

2. Belgium:

Institut Scientifique de la Santé Publique - Louis Pasteur, 14 Rue Juliette Wytsman - B 1050 Bruxelles - Belgique;

3. France:

Laboratoire national des médicaments vétérinaires, Fougeres;

4. Grand Duchy of Luxembourg:

Institute of the supplying country; 5.

5. Italy:

Istituto superiore di sanità, Rome;

6. Netherlands:

Centraal Instituut voor Dierziekte Controle Lelystad (CIDC-Lelystad), Lelystad;

7. Denmark:

Danmarks Veterinærinstitut, Bulowsvej 27, DK-1790 København;

8. Ireland:

Institute of the supplying country;

9. United Kingdom:

Veterinary Laboratory Agency, Addlestone, Weybridge;

10. Greece:

Κετρω ΚΤγνιάρπτια ν Ι8ρπυφΤmv, ΝεανοΑεuc 25, 153 10 ΑΒnvα;

11. Spain:

Laboratorio de Sanidad y producción animal de Granada;

## 12. Portugal:

Laboratorio Nacional de Investigacao Veterinaria, Lisbon;

## 13. Austria:

Bundesanstalt für veterinärmedizinische Untersuchungen, Modling;

## 14. Finland:

Eläinlääkintä - ja elintarviketutkimus - laitos - Forskningsanstalten för veterinärmedicin och livsmedel, Helsinki;

## 15. Sweden:

Statens veterinärmedicinska anstalt, Uppsala.'

## SCHEDULE C

### BRUCELLOSIS

#### 1. IDENTIFICATION OF THE AGENT

The demonstration by modified acid-fast or immunospecific staining of organisms of *Brucella* morphology in abortion material, vaginal discharges or milk provides presumptive evidence of brucellosis, especially if supported by serological tests.

After isolation, the species and biovar should be identified by phage lysis and, or oxidative metabolism tests, cultural, biochemical and serological criteria.

The techniques and media used, their standardisation and the interpretation of results must conform to that specified in the CIE Manual of Standards for Diagnostic Tests and Vaccines, Fourth Edition, 2000, Chapter 2.3.1 (bovine brucellosis), Chapter 2.4.2 (caprine and ovine brucellosis) and Chapter 2.6.2 (porcine brucellosis).

#### 2. IMMUNOLOGICAL TESTS

##### 2.1. Standards

2.1.1. The *Brucella abortus* biovar I Weybridge strain No 99 or USDA strain 1119-3 must be used for the preparation of all antigens used in the rose bengal test (RBT), serum agglutination test (SAT), complement fixation test (CFT) and the milk ring test (MRT).

2.1.2. The standard reference serum for the RBT, SAT, CFT and MRT is the OIE international reference standard serum (OIEISS) formerly named WHO second international anti-*Brucella abortus* Serum (ISAbS).

2.1.3. The standard reference sera for ELISAs are -

- the OIEISS,

- the weak positive OIE ELISA standard serum (OIEELISA<sub>WPSS</sub>),

- the strong positive OIE ELISA standard serum (OIEELISA<sub>SPSS</sub>),

- The negative OIE ELISA standard serum (OIEELISA<sub>N</sub>SS).

2.1.4. The above listed standard sera are available from the Veterinary Laboratories Agency (VLA), Weybridge, United Kingdom.

2.1.5. The OIEISS, the OIEELISA<sub>H,P</sub>SS, the OIEELISA<sub>,,SS</sub> and the OIEELISA<sub>N</sub>SS are international primary standards from which secondary reference national standards ('working standards') must be established for each test in each Member State.

2.2. **Enzyme-linked immunosorbent assays (ELISAs) or other binding assays for the detection of bovine brucellosis in serum or milk**

2.2.1. *Material and reagents*

The technique used and the interpretation of results must have been validated in accordance with the principles laid down in Chapter 1.1.3 of the CIE Manual of Standards for Diagnostic Tests and Vaccines, Fourth Edition, 2000, and should at least include laboratory and diagnostic studies.

2.2.2. *Standardisation of the test*

2.2.2.1. Standardisation of the test procedure for individual serum samples -

(a) a 1/150 pre-dilution <sup>(1)</sup> of the OIEISS or a 1/2 pre-dilution of the OIEELISA<sub>,,SS</sub> or a 1/16 pre-dilution of the OIEELISA<sub>,,SS</sub> made up in a negative serum (or in a negative pool of sera) should give a positive reaction;

(b) a 1/600 pre-dilution of the OIEISS or a 1/8 pre-dilution of the OIEELISA<sub>,,SS</sub> or a 1/64 pre-dilution of the OIEELISA<sub>,,SS</sub> made up in a negative serum (or in a negative pool of sera) should give a negative reaction;

(c) the OIEELISA<sub>N</sub>SS should always give a negative reaction.

2.2.2.2. Standardisation of the test procedure for pooled serum samples -

(a) a 1/150 pre-dilution of the OIEISS or a 1/2 pre-dilution of the OIEELISA<sub>W,P</sub>SS or a 1/16 pre-dilution of the OIEELISA<sub>S</sub>SS made up in a negative serum (or in a negative pool of sera) and again diluted in negative sera by the number of samples making up the pool should give a positive reaction;

(b) the OIEELISA<sub>N</sub>SS should always give a negative reaction;

(c) the test must be adequate to detect evidence of infection in a single animal of the group of animals, of which samples of serum have been pooled.

2.2.2.3. Standardisation of the test procedure for pooled milk or whey samples -

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<sup>1</sup> For the purpose of this Schedule, dilutions given for making up liquid reagents are expressed as, for example, 1 150 shall mean a 1 in 150 dilution.

- (a) a 1/1000 pre-dilution of the OIEISS or a 1/16 pre-dilution of the OIEELISA<sub>SS</sub> or a 1/125 pre-dilution of the OIEELISA<sub>5SS</sub> made up in a negative serum (or in a negative pool of sera) and again diluted 1/10 in negative milk should give a positive reaction;
- (b) the OIEELISA<sub>NSS</sub> diluted 1/10 in negative milk should always give a negative reaction;
- (c) the test must be adequate to detect evidence of infection in a single animal of the group of animals, of which samples of milk or whey have been pooled.

#### 2.2.3. Conditions for use of the ELISAs for diagnosis of bovine brucellosis -

- 2.2.3.1. Using the abovementioned calibrating conditions for ELISAs on serum samples, the diagnostic sensitivity of ELISA shall be equal or greater than the RBT or CFT taking into account the epidemiological situation under which it is employed.
- 2.2.3.2. Using the abovementioned calibrating conditions for ELISA on pooled milk samples, the diagnostic sensitivity of ELISA shall be equal or greater than the MRT taking into account not only the epidemiological situation but also the average and expected extreme husbandry systems.
- 2.2.3.3. Where ELISAs are used for certification purposes in accordance with regulation 6(1) or for the establishment and maintenance of a herd status in accordance with Schedule A(II)(10), pooling of samples of serum must be carried out in such a way that the test results can be undoubtedly related to the individual animal included in the pool. Any confirmatory test must be carried out on samples of serum taken from individual animals.
- 2.2.3.4. The ELISAs may be used on a sample of milk taken from the milk collected from a farm with at least 30 % of dairy cows in milk. If this method is used, measures must be taken to ensure that the samples taken for examination can be undoubtedly related to the individual animals from which the milk derived. Any confirmatory test must be carried out on samples of serum taken from individual animals.

#### 2.3. Complement fixation test (CFT)

- 2.3.1. The antigen represents a bacterial suspension in phenol-saline (NaCl 0,85 % (m/v) and phenol at 0,5 % (v/v)) or in a veronal buffer. Antigens may be delivered in the concentrated state provided the dilution factor to be used is indicated on the bottle label. The antigen must be stored at 4 °C and not frozen.
- 2.3.2. Serums must be inactivated as follows -
  - bovine serum: 56 to 60 °C for 30 to 50 minutes,
  - porcine serum: 60 °C for 30 to 50 minutes.
- 2.3.3. In to carry out the genuine reaction within the test procedure, a complement dose higher than the minimum necessary for total haemolysis should be used.

2.3.4. In carrying out the complement fixation test, the following controls must be made each time -

- (a) control of the anti-complementary effect of the serum;
- (b) control of the antigen;
  
- (c) control of sensitised red blood cells;
  
- (d) control of the complement;
  
- (e) control using a positive serum of sensitivity at the start of the reaction; (I) control of the specificity of the reaction using a negative serum.

2.3.5. Calculation of results

The OIEISS contains 1 000 international CFT units (ICFTU) per ml. If the OIEISS~ is tested in a given method the result is given as a titre ( $T_{\text{OIEISS}}$ ). The test result for the test serum given as titre ( $T_{\text{TESTSERUM}}$ ) must be expressed in ICFTU per ml. In order to convert the expression of a titre into ICFTU, the factor (F) necessary to convert a titre of an unknown test serum ( $T_{\text{TESTSERUM}}$ ) tested by that method into the ICFTU expression can be found from the Formula -

$$F = 1\,000 \times 1 / T_{\text{OIEISS}}$$

and the content of international CFT units per ml of test serum ( $ICFTU_{\text{TESTSERUM}}$ ) from the formula -

$$ICFTU_{\text{TESTSERUM}} = F \times T_{\text{TESTSERUM}}$$

2.3.6. Interpretation of results

A serum containing 20 or more *ICFTU* per ml is considered to be positive.

#### 2.4. Milk ring test (MRT)

2.4.1. The antigen represents a bacterial suspension in phenol-saline (NaCl 0,85 % (m/v) and phenol at 0,5 % (v/v)) stained with haematoxylin. The antigen must be stored at 4°C and not frozen.

2.4.2. The antigen sensitivity must be standardised in relation to the OIEISS in such a way that the antigen produces a positive reaction with a 1/500 dilution of the OIEISS in negative milk, while a 1/1 000 dilution should be negative.

2.4.3. The ring test must be made on samples representing the contents of each milk chum or the content of each bulk tank from the farm.

2.4.4. The milk samples must not have been frozen, heated or subjected to violent shaking.

2.4.5. The reaction must be carried out using one of the following methods -

- on a column of milk of at least 25 mm height and on a volume of milk of 1 ml to which either 0,03 ml or 0,05 ml of one of the standardised stained antigens has been added,

- on a column of milk of at least 25 mm height and on a volume of milk of 2 ml to which 0,05 ml of one of the standardised stained antigens has been added,

- on a volume of milk of 8 ml to which 0,08 ml of one of the standardised stained antigens has been added.

2.4.6. The mixture of milk and antigens must be incubated at 37 °C for 60 minutes, together with positive and negative working standards. A subsequent 16 to 24 hour incubation at 4 °C increases the sensitivity of the test.

2.4.7. Interpretation of results -

(a) negative reaction: coloured milk,  
colourless cream;

(b) positive reaction -

- identically coloured milk  
and cream, or

- colourless milk and  
coloured cream.

## 2.5. **Rose bengal plate Test (RBT)**

2.5.1. The antigen represents a bacterial suspension in buffered Brucella antigen diluent at a pH of 3,65 ± 0,05, stained by the use of rose bengal dye. The antigen shall be delivered ready for use and must be stored at 4 °C and not frozen.

2.5.2. The antigen shall be prepared without reference to the cell concentration, but its sensitivity must be standardised in relation to the OIEISS in such a way that the antigen produces a positive reaction with a serum dilution of 1/45 and a negative reaction with a dilution of 1/55.

2.5.3. The RBT shall be carried out in the following manner -

(a) serum (20-30 pd) is mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter. The mixture is rocked gently for 4 minutes at ambient temperature, and then observed in a good light for agglutination;

(b) an automated method may be used but must be at least as sensitive and accurate as the manual method.

2.5.4. Interpretation of results

Any visible reaction is considered to be positive, unless there has been excessive drying round the edges. Positive and negative working standards should be included in each series of tests.

## 2.6. **Serum agglutination test (SAT)**

2.6.1. The antigen represents a bacterial suspension in phenol-saline (NaCl 0,85 % (m/v) and phenol at 0,5 % (v/v)). Formaldehyde must not be used.

Antigens may be delivered in the concentrated state provided the dilution factor to be used is indicated on the bottle label.

EDTA may be added to the antigen suspension to 5 mM final test dilution to reduce the level of false positives to the serum agglutination test. Subsequently the pH of 7,2 must be readjusted in the antigen suspension.

2.6.2. The OIEISS contains 1 000 international units of agglutination.

2.6.3. The antigen shall be prepared without reference to the cell concentration, but its sensitivity must be standardised in relation to the OIEISS in such a way that the antigen produces either a 50 % agglutination with a final serum dilution of 1/600 to 1/1 000 or a 75 % agglutination with a final serum dilution of 1/500 to 1/750.

It may also be advisable to compare the reactivity of new and previously standardised batches of antigen using a panel of defined sera.

2.6.4. The test is performed either in tubes or in microplates. The mixture of antigen and serum dilutions should be incubated for 16 to 24 hours at 37°C.

At least three dilutions must be prepared for each serum. Dilutions of suspect serum must be made in such a way that the reading of the reaction at the positivity limit is made in the median tube (or well for the microplate method).

2.6.5. Interpretation of results:

The degree of Brucella agglutination in a serum must be expressed in IU per ml.

A serum containing 30 or more IU per ml is considered to be positive.

### 3. COMPLEMENTARY TESTS

#### 3.1. Brucellosis skin test (BST)

##### 3.1.1. Conditions for the use of BST

(a) The brucellosis skin test shall not be used for the purpose of certification for Intra-Community trade.

(b) The brucellosis skin test is one of the most specific tests for the detection of brucellosis in unvaccinated animals, however diagnosis should not be made on the basis of positive intradermal reactions alone.

(c) Bovine animals, tested with negative result in one of the serological tests defined in this Annex and reacting positively to the BST shall be regarded as infected.

(d) Bovine animals, tested with positive result in one of the serological tests defined in this Annex may be subject to a BST in to support the interpretation of the serological test results, in particular where in brucellosis free or officially free herds a cross-reaction with antibodies against other bacteria cannot be excluded.

3.1.2. The test must be carried out by use of a standardised and defined brucellosis allergen preparation that does not contain smooth lipopolysaccharide (LPS) antigen, as this may provoke non-specific inflammatory reactions or interfere with subsequent serological tests.

One of such preparation is Brucellin INRA prepared from a non smooth strain of *B. melitensis*. The requirements for its production are detailed in Section B2 of Chapter 2.4.2. of the OIE Manual of Standards for Diagnostic Tests and Vaccines, Fourth Edition, 2000.

##### 3.1.3. Test procedure

3.1.3.1. A volume of 0,1 ml of brucellosis allergen is injected intradermally into the caudal fold, the skin of the flank, or the side of the neck.

3.1.3.2. The test is read after 48-72 hours.

3.1.3.3. The skin thickness at the injection site is measured with vernier callipers before injection and at re-examination.

3.1.3.4. Interpretation of results -

Strong reactions are easily recognised by local swelling and induration.

Skin thickening of 1,5 to 2 mm shall be considered as positive reaction to the BST.

### 3.2. **Competitive enzyme-linked immunosorbent assay (cELISA)**

3.2.1. Conditions *for the* use of cELISA

(a) The cELISA shall not be used for the purpose of certification for intra-Community trade.

(b) The cELISA has shown to have a higher specificity than for example the indirect ELISA and may therefore be used in to support the interpretation of the serological test results.

3.2.2. Test *procedure*

The test shall be carried out in accordance with the prescription in the CIE Manual of Standards for Diagnostic Tests and Vaccines, Fourth Edition, 2000, Chapter 2.3.1(2)(a).

## 4. **NATIONAL REFERENCE LABORATORIES**

### 4.1. **Tasks and responsibilities**

National reference laboratories shall be responsible for -

(a) the approval of the results of the validation studies demonstrating the reliability of the test method used in the Member State;

(b) determination of the maximum number of samples to be pooled in ELISA kits used;

(c) calibration of the standard secondary reference national standard sera ('working standards) against the primary international standard serum referred to in paragraph 2.1;

(d) quality checks of all antigens and ELISA kits batches used in the Member State;

(e) cooperation within the European Union Network of National Reference Laboratories for Brucellosis.

### 4.2. **List of national reference laboratories**

BELGIUM

Centre d'etudes et de recherches veterinaires et agrochimiques (CERVA/CODA) Groeselenberg 99  
B-1180 Bruxelles/Brussel

B 8444

DENMARK

Danish Veterinary Institute Bulowsvej 27 DK-1790 Copenhagen

GERMANY

Bundesinstitut für Gesundheitlichen Verbraucherschutz and Veterinärmedizin (BGVV) Nationales Veterinärmedizinisches Referenzlabor für Brucellose Postfach 33 00 13 D-14191 Berlin

GREECE

Veterinary Laboratory of Larissa Department of Microbiology  
6th km of National Road Larissa-Trikala GR-4111 10 Larissa

SPAIN

Laboratorio Central de Veterinaria de Santa Fe Camino del Jau S/N  
E-18320 Santa Fe (Granada)

FRANCE

Laboratoire national et OIE/FAO de référence pour la brucellose Agence française de sécurité sanitaire  
des aliments (AFSSA) BP 67  
F-94703 Maisons-Alfort Cedex

IRELAND

Brucellosis Laboratory Model Farm Road Cork  
Ireland

ITALY

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise Via Campo Boario  
1-64100 Teramo

LUXEMBOURG

State Laboratory for Veterinarian Medicine 54, avenue Gaston Diderich B.P. 2081  
L-1020 Luxembourg

NETHERLANDS

Centraal Instituut voor Dierziektecontrole CIDC-Lelystad  
Houtribweg 39 P.O. Box 2004 8203 AA Lelystad Nederland

AUSTRIA

Bundesanstalt für veterinärmedizinische Untersuchungen Robert-Koch-Gasse 17  
A-2340 Modling

PORTUGAL

Laboratorio Nacional de Investigação Veterinária (LNIV) Estrada de Benfica, n.º 701 P-1549-011 Lisboa

FINLAND

National Veterinary and Food Research Institute Hameentie 57  
PO Box 45  
FIN-00581 Helsinki

SWEDEN

National Veterinary Institute S-751 89 Uppsala

UNITED KINGDOM

1. FAO/WHO Collaborating Centre for Reference and Research on Brucellosis Veterinary  
Laboratories Agency  
New Haw  
Addlestone

Surrey KT15 3NB  
United Kingdom

2. Immunodiagnostics Department Veterinary Sciences Division Stoney Road Stormont Belfast BT4 3SD  
United Kingdom'

## **SCHEDULE D**

### **CHAPTER I**

#### **OFFICIALLY ENZOOTIC-BOVINE-LEUKOSIS-FREE HERDS, MEMBER STATES AND REGIONS**

A. Officially enzootic-bovine-leukosis-free herd means a herd in which -

- (i) there is no evidence, either clinical or as a result of a laboratory test, of any case of enzootic bovine leukosis in the herd and no such case has been confirmed in the previous two years; and
- (ii) all animals over 24 months of age have reacted negatively during the preceding 12 months to two tests carried out in accordance with this Annex, at an interval of at least four months; or
- (iii) it meets the requirements of (i) above and is situated in an officially enzootic-bovine-leukosis-free Member State or region.

B. A herd shall retain officially enzootic-bovine-leukosis-free status provided -

- (i) the condition in A(i) continues to be fulfilled;
- (ii) any animals introduced into the herd come from an officially enzootic-bovine-leukosis-free herd;
- (iii) all animals over 24 months of age continue to react negatively to a test carried out in accordance with Chapter II at intervals of three years;
- (iv) breeding animals introduced into a herd and originating from a third country have been imported in accordance with European Union Council Directive 72/462/EEC.

C. The officially leukosis-free status of a herd is to be suspended if the conditions detailed in B are not fulfilled, or where as a result of laboratory tests or on clinical grounds one or more bovine animals are suspected of having enzootic bovine leukosis and the suspect animal(s) are immediately slaughtered.

D. The status is to remain suspended until the following requirements are complied with -

1. If a single animal in an officially enzootic-bovine-leukosis-free herd has reacted positively to one of the tests referred to in Chapter II, or where infection is otherwise suspected in one animal in a herd -

- (i) the animal which has reacted positively, and, in the case of a cow, any calf it may have produced, must have left the herd for slaughter under the supervision of the veterinary authorities;
- (ii) all animals in the herd more than 12 months old have reacted negatively to two serological tests (at least 4 months and less than 12 months apart) carried out in

accordance with Chapter II three months at least after removal of the positive animal and any possible progeny thereof;

(iii) an epidemiological inquiry has been conducted with negative results and the herds linked epidemiologically to the infected herd have been subjected to the measures laid down in (ii). However, the competent authority may grant a derogation from the obligation to slaughter the calf of an infected cow where it was separated from its mother immediately after calving. In this case, the calf must be made subject to the requirements provided for in 2(iii).

2. Where more than one animal from an officially enzootic-bovine-leukosis-free herd has reacted positively to one of the tests referred to in Chapter II, or where infection has otherwise been suspected in more than one animal in a herd -

(i) any animals which have reacted positively and, in the case of cows, their calves, must be removed for slaughter under the supervision of the veterinary authorities;

(ii) all animals in the herd aged over 12 months must react negatively to two tests carried out in accordance with Chapter II at an interval of at least four months and no more than 12 months;

(iii) all other animals in the herd must, after identification, remain on the holding until they are aged over 24 months and have been tested in accordance with Chapter II after reaching that age, except that the competent authority may permit such animals to go directly for slaughter under official supervision;

(iv) an epidemiological inquiry has been conducted with negative results and any herd linked epidemiologically to the infected herd has been subjected to the measures laid down in (ii). However, the competent authority may grant a derogation from the obligation to slaughter the calf of an infected cow where it was separated from its mother immediately after calving. In this case, the calf must be made subject to the requirements provided for in 2(iii).

E. In accordance with the procedure in Article 17 of the European Union Council Directive 64/432/EEC and on the basis of information supplied in accordance with regulation 8, a Member State or part of a Member State may be considered officially enzootic-bovine leukosis-free if -

(a) all the conditions of paragraph A are fulfilled and at least 99,8 % of the bovine herds are officially enzootic-bovine-leukosis-free;

or

(b) no case of enzootic bovine leukosis has been confirmed in the Member State or the part of the Member State for the past three years, and the presence of tumours suspected of being due to EBL is compulsorily notifiable, with investigations of cause being carried out, and in the case of a Member State, all animals aged over 24 months in at least 10 % of the herds, selected randomly, have been tested with negative results in accordance with Chapter II in the previous 24 months, or in the case of a part of a Member State, all animals aged over 24 months have undergone a test provided for in Chapter II with negative results in accordance with Chapter II in the previous 24 months;

or

(c) any other method which demonstrates to a confidence rating of 99 % that less than 0,2 % of herds were infected.

F. A Member State or a region of a Member State is to retain officially enzootic-bovine-leukosis-free status if

- (a) all animals slaughtered within the territory of that Member State or region are submitted to official post-mortem examinations at which all tumours which could be due to the EBL virus are sent for laboratory examination,
- (b) the Member State reports to the European Commission all cases of enzootic bovine leukosis that occur in the region,
- (c) all animals which react positively to any of the tests provided for in Chapter II are slaughtered and their herds remain subject to restrictions until re-establishment of their status in accordance with Section D, and
- (d) all animals more than two years old have been tested, either once in the first five years after the status is granted under Chapter II or during the first five years after the grant of the status under any other procedure demonstrating with a certainty level of 99 % that less than 0,2 % of herds have been infected.

However, where no case of enzootic bovine leukosis has been recorded in a Member State or in a region of a Member State in a proportion of one herd out of 10 000 for at least three years, a decision may be taken in accordance with the procedure laid down in Article 17 of the European Union Council Directive 64/432/EEC that routine serological tests may be reduced provided that all bovine animals more than 12 months old in at least 1 % of herds, selected at random each year, have been subjected to a test carried out in accordance with Chapter II.

G. The officially enzootic-bovine-leukosis-free status of a Member State or part of a Member State is to be suspended, in accordance with the procedure in Article 17 of the European Union Council Directive 64/432/EEC if, as a result of investigations carried out in accordance with paragraph F above, there is evidence of a significant change in the situation as regards enzootic bovine leukosis in a Member State or part of a Member State which has been recognised as officially enzootic-bovine-leukosis-free.

The officially enzootic-bovine-leukosis-free status may be restored in accordance with the procedure in regulation 17 when the criteria laid down by the same procedure are fulfilled.

## CHAPTER II

### TESTS FOR ENZOOTIC BOVINE LEUKOSIS

Tests for enzootic bovine leukosis shall be carried out by the immunediffusion test under the conditions described in A and B or by the enzymelinked immunosorbent assay (ELISA) under the conditions described in C. The immune diffusion method may only be used for individual tests. If test results are the subject of a duly substantiated challenge, an additional check shall be carried out by means of the immunediffusion test.

A. Agar gel immunediffusion test for enzootic bovine leukosis

1. The antigen to be used in the test must contain bovine leukosis virus glycoproteins. The antigen must be standardized against a standard serum (EI serum) supplied by the State Veterinary Serum Laboratory, Copenhagen.
2. The official institutes indicated below must be made responsible for calibrating the standard working antigen of the laboratory against the official EEC standard serum (EI serum) provided by the State Veterinary Serum Laboratory, Copenhagen.

- (a) Germany:  
Bundesforschungsanstalt für Viruskrankheiten der Tiere, Tübingen;

- (b) Belgium:  
Institut national de recherches vétérinaires, Brussels;
- (c) France:  
Laboratoire national de pathologie bovine, Lyon;
- (d) Grand Duchy of Luxembourg:
- (e) Italy:  
Istituto zooprofilattico sperimentale, Perugia;
- (f) Netherlands:  
Instituut voor Dierhouderij en Diergezondheid (IDDLO), Lelystad;
- (g) Denmark:  
Statens Veterinære Serum Laboratorium, Copenhagen;
- (h) Ireland:  
Veterinary Research Laboratory, Abbotstown, Dublin;
- (i) United Kingdom:
- (1) Great Britain:  
Veterinary Laboratory Agency, Addlestone, Weybridge, England;
- (2) Northern Ireland:  
Veterinary Research Laboratory, Stormont, Belfast;
- (j) Spain:  
Subdirección general de sanidad animal. Laboratorio de sanidad y producción animal ALGETE, Madrid;
- (k) Portugal:  
Laboratório Nacional de Investigação Veterinária, Lissabon;
- (l) Greece: Κέντρο Κτηνιατρικών Ιδρυμάτων Νεαπόλεως 25, 15310 Αθήνα;;
- (m) Austria:  
Bundesanstalt für Tierseuchenbekämpfung, Mödling;
- (n) Finland:  
eläinlääkintä ja elintarvikelaitos, Helsinki/anstalten för veterinärmedicin och livsmedel, Helsingfors;
- (o) Sweden:  
Statens veterinärmedicinska anstalt, Uppsala.

3. The standard antigens used in the laboratory must be submitted at least once a year to the EEC reference laboratories listed in 2 for testing against the official EEC standard serum. Apart from this standardization, the antigen in use can be calibrated in accordance with B.

4. The reagents for the test shall consist of -

- (a) antigen: the antigen must contain specific glycoproteins of enzootic bovine leukosis virus which has been standardized against the official EEC serum;

(b) the test serum;

(c) known positive control serum;

(d) agar gel:

0, 8% agar, 8, 5 % NaCl, 0, 05 M Trisbuffer pH 7, 2; 15 ml of this agar must be introduced into a petri dish of 85 mm diameter, resulting in a depth of 2, 6 mm of agar.

5. A test pattern of seven moisture free wells must be cut in the agar to the bottom of the plate; the pattern must consist of one central well and six wells in a circle around it.

Diameter of central well: 4 mm

Diameter of peripheral wells: 6 mm

Distance between central and peripheral wells: 3 mm

6. The central well must be filled with the standard antigen. Peripheral wells 1 and 4 (see diagram below) are filled with the known positive serum, wells 2, 3, 5 and 6 with the test sera. The wells must be filled until the meniscus disappears.

7. This results in the following quantities being obtained -

antigen: 32  $\mu$ l

control serum: 73  $\mu$ l

test serum: 73  $\mu$ l.

8. Incubation must be for 72 hours at room temperature (20 to 27°C) in a closed humid chamber.

9. The test may be read at 24 and 48 hours but a final result may not be obtained before 72 hours:

(a) a test serum is positive if it forms a specific precipitin line with the BLV antigen and forms a complete line of identity with the control serum;

(b) a test serum is negative if it does not form a specific precipitin line with the BLV antigen and if it does not bend the line of the control serum;

(c) the reaction cannot be considered conclusive if it -

(i) bends the line of the control serum towards the BLV antigen well without forming a visible precipitin line with the antigen;

or

(ii) if it cannot be read either as negative or as positive. In inconclusive reactions the test may be repeated and concentrated serum utilized.

10. Any other well configuration or pattern may be utilized provided that the E4 serum diluted 1:10 in negative serum can be detected as positive.

## B. Method for antigen standardization

Solutions and materials required

B 8450

1. 40 ml of 1, 6 % agarose in 0, 05 M Tris/HCl buffer, pH 7, 2 with 8, 5 % NaCl;
2. 15 ml of a bovine leukosis serum, having antibody only to bovine leukosis virus glycoproteins, diluted 1:10 in 0, 05 M Tris/HCl buffer, pH 7, 2 with 8, 5 % NaCl;
3. 15 ml of a bovine leukosis serum, having antibody only to bovine leukosis virus glycoproteins, diluted 1:5 in 0, 05 M Tris/HCl buffer, pH 7, 2 with 8, 5 % NaCl;
4. four plastic petri dishes with a diameter of 85 mm;
5. a punch with a diameter of 4 to 6 mm;
6. a reference antigen;
7. the antigen which is to be standardized;
8. a water bath (56°C).

*Procedure*

Dissolve the agarose (1, 6 %) in the Tris/HCl buffer by carefully heating to 100°C. Place in WC water bath for approximately one hour. Also, place the bovine leukosis serum dilutions in a WC water bath.

Now mix 15 ml of the 56°C agarose solution with the 15 ml bovine leukosis serum (1:10), quickly shake and pour 15 ml into each of two petri dishes. Repeat this procedure with the bovine leukosis serum diluted 1:5,

When the agarose has hardened, holes are made in it as follows -

Addition of antigen

(i) Petri dishes 1 and 3:

well A undiluted reference antigen;  
well B 1:2 diluted reference antigen;  
wells C and E reference antigen;  
well D undiluted test antigen.

(ii) Petri dishes 2 and 4:

well A undiluted test antigen;  
well B 1:2 diluted test antigen;  
well C 1:4 diluted test antigen;  
well D 1:8 diluted test antigen.

*Additional instructions*

1. The experiment shall be carried out with two serum dilutions (1:5 and 1:10) in to achieve optimal precipitation
2. If the precipitation diameter is too small with both dilutions, then the serum must be further diluted.
3. If the precipitation diameter in both dilutions is too large and faint, then a lower serum must be chosen.

4. The final concentration of the agarose must be 0, 8%; that of the sera 5 and 10% respectively.
5. Plot the measured diameters in the following co-ordinate system. The dilution of the antigen to be tested with the same diameter as the reference antigen is the working dilution.

C. Enzymelinked immunosorbent assay (ELISA) for detecting enzootic bovine leukosis

1. The material and reagents to be used are as follows -

- (a) solidphase microplates, cuvettes or any other solid phase;
- (b) the antigen is fixed to the solid phase with or without the aid of polyclonal or monoclonal catching antibodies. If antigen is coated directly to the solid phase, all test samples giving positive reactions have to be retested against control antigen in the case of EBL. The control antigen should be identical to the antigen except for the BLV antigens. If catching antibodies are coated to the solid phase, the antibodies must not react to antigens other than BLV antigens;
- (c) the biological fluid to be tested;
- (d) a corresponding positive and negative control;
- (e) conjugate;
- (f) a substrate adapted to the enzyme used;
- (g) a stopping solution, if necessary;
- (h) solutions for the dilution of the test samples for preparations of the reagents and for washing;
- (i) a reading system appropriate to the substrate used..

2. Standardization and sensitivity of test The sensitivity of the ELISA assay must be of such a level that E4 serum is scored positive when diluted 10 times (serum samples) or 250 times (milk samples) more than the dilution obtained of individual samples when these are included in pools. In assays where samples (serum and milk) are tested individually E4 serum diluted 1 to 10 (in negative serum) or 1 to 250 (in negative milk) must be scored positive when tested in the same assay dilution as used for the individual test samples. The official institutes listed in A.2 will be responsible for checking the quality of the ELISA method, and in particular for determining, for each production batch, the number of samples to be pooled on the basis the count obtained for the E4 serum.

The E4 serum will be supplied by the National Veterinary Laboratory, Copenhagen.

3. Conditions for use of the ELISA test for EBL The Elisa method may be used on a sample of milk or whey taken from the milk collected from a farm with at least 30 % of dairy cows in milk. If this method is used, measures must be taken to ensure that the samples taken can be identified with the animals from which the milk or sera examined were taken.

**SCHEDULE E**  
**(I)**

(a) Bovine diseases

- Foot-and-mouth disease
- Rabies
- Tuberculosis
- Brucellosis
- Contagious bovine pleuropneumonia
- Enzootic bovine leukosis
- Anthrax

(b) Swine diseases

- Rabies
- Brucellosis
- Classical swine fever African swine fever
- Foot-and-mouth disease
- Swine vesicular disease
- Anthrax

**SCHEDULE E**  
**(II)**

- Aujeszky's disease
- Infectious bovine rhinotracheitis
- Brucella suis infection
- Transmissible gastroenteritis.

**SCHEDULE F**

Where in these regulations reference is made to Schedule F of these regulations or Annex F of the European Union Council Directive 64/432/EEC reference should be made to the European Union Commission Regulation (EC) No 599/2004 concerning the adoption of a harmonised model certificate and inspection report linked to intra-Community trade in animals and products of animal origin