COMMISSION REGULATION (EC) No 535/2002
of 21 March 2002
amending Annex C to Council Directive 64/432/EEC and amending Decision 2000/330/EC

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine [1], as last amended by Commission Decision 2001/298/EC [2], and in particular Article 16(1), second paragraph thereof,

Whereas:

(1) On 11 October 1999 the Scientific Committee on Animal Health and Animal Welfare adopted a report [3] on the modification of technical Annexes to Directive 64/432/EEC to take account of scientific developments regarding tuberculosis, brucellosis and enzootic bovine leucosis.

(2) In accordance with the above report, the tests for brucellosis should be carried out in line with the Manual of Standards for Diagnostic Tests and Vaccines, Third Edition, 1996, of the Office International des Epizooties (OIE).

(3) In August 2001 the OIE published the Fourth Edition 2000 of the said Manual, including certain modifications in the description of tests for brucellosis.

(4) It was therefore necessary to amend Annex C to Directive 64/432/EEC so as to lay down test procedures applicable for surveillance and trade purposes within the Community which reflect as much as possible the OIE standards but take also into account the advice of the Scientific Committee and of the National Reference Laboratories in the Member States cooperating within the framework of the European Union Network of National Reference Laboratories for Brucellosis.

(5) Commission Decision 2000/330/EC of 18 April 2000 approving tests for the detection of antibodies against bovine brucellosis within the framework of Council Directive 64/432/EEC [4] should be amended accordingly.

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

HAS ADOPTED THIS REGULATION:

Article 1

Annex C to Directive 64/432/EEC is replaced by the Annex to the present Regulation.

Article 2

Decision 2000/330/EC is amended as follows:

1. Article 1 is replaced by the following:

   '*Article 1*

   The complement fixation test, the buffered brucella antigen tests and the ELISA tests carried out in accordance with the provisions in Annex C to Directive 64/432/EEC are hereby approved for certification purposes.'

2. The Annex is deleted.

Article 3

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

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 21 March 2002.

For the Commission

David BYRNE

Member of the Commission

[1] OJ 121, 29.7.1964, p. 1977/64.
[2] OJ L 102, 12.4.2001, p. 63.
[3] SANCO/B3/R10/1999.
[4] OJ L 114, 13.5.2000, p. 37.

ANNEX

'ANNEX 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 OIE 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 1 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 ($OIEELISA_{WP}SS$),

— the strong positive OIE ELISA standard serum ($OIEELISA_{SP}SS$),

— The negative OIE ELISA standard serum ($OIEELISA_{N}SS$).

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 $OIEELISA_{WP}SS$, the $OIEELISA_{SP}SS$ and the $OIEELISA_{N}SS$ 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 OIE 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 ([1]) of the OIEISS or a 1/2 pre-dilution of the $OIEELISA_{WP}SS$ or a 1/16 pre-dilution of the $OIEELISA_{SP}SS$ 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 $OIEELISA_{WP}SS$ or a 1/64 pre-dilution of the $OIEELISA_{SP}SS$ made up in a negative serum (or in a negative pool of sera) should give a negative reaction;

([1]) For the purpose of this Annex, dilutions given for making up liquid reagents are expressed as, for example, 1/150 shall mean a 1 in 150 dilution.

(c) the OIEELISA$_N$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$_{WP}$SS or a 1/16 pre-dilution of the OIEELISA$_{SP}$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$_N$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:

(a) a 1/1000 pre-dilution of the OIEISS or a 1/16 pre-dilution of the OIEELISA$_{WP}$SS or a 1/125 pre-dilution of the OIEELISA$_{SP}$SS 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$_N$SS 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 Article 6(1) or for the establishment and maintenance of a herd status in accordance with Annex 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 order 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;

(f) control of the specificity of the reaction using a negative serum.