[F1[F2[F3ANNEX C

BRUCELLOSIS

Textual Amendments

F1 Substituted by Council Directive 97/12/EC of 17 March 1997 amending and updating Directive 64/432/EEC on health problems affecting intra-Community trade in bovine animals and swine.

F2 Substituted by 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.

F3 Substituted by Commission Decision of 10 December 2008 amending Annex C to Council Directive 64/432/EEC and Decision 2004/226/EC as regards diagnostic tests for bovine brucellosis (notified under document number C(2008) 7642) (Text with EEA relevance) (2008/984/EC).

2. IMMUNOLOGICAL TESTS

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 1.1.4 of the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Sixth Edition, 2008, and must include at least 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) must 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) must give a negative reaction;

(c) the OIEELISA$_{N}$SS must 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 must give a positive reaction;

(b) the OIEELISA$_{N}$SS must 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 1/1 000 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 must give a positive reaction;

(b) the OIEELISA$_{N}$SS diluted 1/10 in negative milk must 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 calibrating conditions for ELISAs set out in point 2.2.2.1 and 2.2.2.2 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 calibrating conditions for ELISA set out in point 2.2.2.3 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 that 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.**]]]**

(1) **[**[F1]**[**[F2]**[**[F3]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.**]]]**

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