[F1[F1ANNEX D]

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.

F1 Substituted by Council Directive 98/46/EC of 24 June 1998 amending Annexes A, D (Chapter I) and F to Directive 64/432/EEC on health problems affecting intra-Community trade in bovine animals and swine.

[F2CHAPTER II

TESTS FOR ENZOOTIC BOVINE LEUKOSIS

C. Enzyme-linked immunosorbent assay (ELISA) for detecting enzootic bovine leukosis

1. The material and reagents to be used shall be as follows:

(a) solid-phase 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 the control antigen. The control antigen should be identical to the antigen except that the BLV antigens are absent. If catching antibodies are coated to the solid phase, the antibodies shall 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. Standardisation and sensitivity of test

The sensitivity of the ELISA shall be of such a level that the E05 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, the E05 serum diluted 1 to 10 (in negative serum) or 1 to 250 (in negative milk) shall be scored positive when tested in the same assay dilution as used for the individual test samples. The institutes referred to in point 2 of Section A shall be responsible for checking the quality of the ELISA, and in particular for determining, for each production batch, the number of samples to be pooled on the basis of the count obtained for the E05 serum.

3. Conditions for use of the ELISA for enzootic bovine leukosis

(a) ELISAs may be used on serum and milk samples.

(b) Where ELISAs are used for certification purposes in accordance with Article 6(2)(c) or for the establishment and maintenance of a herd status in accordance with Annex D(I), pooling of samples of serum or milk shall be carried out in such a way that the samples taken for examination can be undoubtedly related to the individual animals included in the pool. Any confirmatory test shall be carried out on samples taken from individual animals.

(c) Where ELISAs are used on a sample of bulk milk this sample shall be taken from the milk collected from a herd with at least 30 % of dairy cows in milk. Any confirmatory test shall be carried out on samples of serum or milk taken from individual animals.**]]**

Textual Amendments

F2 Substituted by Commission Decision of 15 December 2009 amending Annex D to Council Directive 64/432/EEC as regards diagnostic tests for enzootic bovine leukosis (notified under document C(2009) 9951) (Text with EEA relevance) (2009/976/EU).