[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

A. Agar gel immuno-diffusion test for enzootic bovine leukosis

1. The antigen to be used in the test shall contain bovine leukosis virus glycoprotein. The antigen shall be standardised against the E05 serum.

2. The State institutes, national reference laboratories or official institutes designated in accordance with Article 6a for coordinating standards and methods of diagnosis of the tests for enzootic bovine leukosis shall be made responsible for calibrating the standard working antigen of the laboratory against the E05 serum.

3. The standard antigens used in the laboratory shall be submitted at least once a year to the State institutes, national reference laboratories or official institutes designated in accordance with Article 6a, for testing against the E05 serum. Apart from such standardisation, the antigen in use may be calibrated in accordance with the method described in Section B.

4. The reagents of the tests shall consist of:

(a) antigen: the antigen shall contain specific glycoprotein of enzootic bovine leukosis virus which has been standardised against the E05 serum;

(b) the test serum;

(c) known positive control serum;

(d) agar gel:
- 0,8 % agar,
- 8,5 % NaCl,
- 0,05 M Tris-buffer pH 7,2,
- 15 ml of this agar shall 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 shall be cut in the agar to the bottom of the plate; the pattern shall 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 shall be filled with the standard antigen. Peripheral wells 1 and 4 described in B.3 are filled with the known positive serum; wells 2, 3, 5 and 6 with the test sera. The wells shall be filled until the meniscus disappears.

7. This results in the following quantities being obtained:

— antigen: 32 µl,

— control serum: 73 µl,

— test serum: 73 µl.

8. Incubation shall 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 shall not be obtained before 72 hours:

(a) a test serum is positive if it forms a specific precipitation line with the bovine leukosis virus (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 precipitation 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 utilised.

10. Any other well configuration or pattern may be utilised provided that the E05 serum diluted 1:10 in negative serum can be detected as positive.**]]**

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).