[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

2.3. 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 shall 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.

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 (i.e. highest direct dilution of the OIEISS giving 50 % haemolysis, T_{OIEISS}). The test result for the test serum given as titre ($T_{TESTSERUM}$) 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 ($T_{TESTSERUM}$) tested by that method into the ICFTU expression can be found from the formula:

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

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

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

2.3.6. Interpretation of results

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