COMMISSION REGULATION (EC) No 1226/2002
of 8 July 2002
amending Annex B to Council Directive 64/432/EEC

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 64/432/EEC [1] of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine, as last amended by Commission Regulation No (EC) 535/2002 [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 of Council 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 tuberculosis 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 tuberculosis.

(4) In January 2002 the European Directorate for the Quality of Medicines published the Fourth Edition 2002 of the European Pharmacopoeia including the monogaphs 0535 and 0536 for the Tuberculin Purified Protein Derivative Avian and Bovine.

(5) It is therefore necessary to amend Annex B to Directive 64/432/EEC so as to lay down test procedures applicable for surveillance and trade purposes within the Community taking account of the opinion of the Scientific Veterinary Committee.

(6) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health,

HAS ADOPTED THIS REGULATION:

Article 1

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

Article 2

This Regulation shall enter into force on the 20th day following that of 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, 8 July 2002.

For the Commission
David BYRNE
Member of the Commission

[1] OJ 121, 29.7.1964, p. 1977/64.
[2] OJ L 80, 23.3.2002, p. 22.
[3] SANCO/B3/R10/1999.

ANNEX

'ANNEX B

TUBERCULOSIS

1. IDENTIFICATION OF THE AGENT

The presence of *Mycobacterium bovis (M. bovis)*, agent of bovine tuberculosis, in clinical and post-mortem specimens may be demonstrated by examination of stained smears or immunoperoxidase techniques and confirmed by cultivation of the organism on primary isolation medium.

Pathological material for the confirmation of *M. bovis* should be taken from abnormal lymph nodes and parenchymatous organs such as lungs, liver, spleen, etc. In the cases where the animal does not present pathological lesions, samples from the retropharyngeal, bronchial, mediastinal, supramammary, mandibular and some mesenteric lymph nodes and liver should be collected for examination and culture.

Identification of isolates may be usually carried out by determining cultural and biochemical properties. The polymerase chain reaction (PCR) may also be employed for the detection of the *M. tuberculosis* complex. DNA analysis techniques may prove to be faster and more reliable than biochemical methods for the differentiation of *M. bovis* from other members of the *M. tuberculosis* complex. Genetic fingerprinting allows distinguishing between different strains of *M. bovis* and will enable patterns of origin, transmission and spread of *M. bovis* to be described.

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 2.3.3 (bovine tuberculosis).

2. THE TUBERCULIN SKIN TEST

Tuberculin PPD (Purified Protein Derivatives) that fulfil the standards laid down in paragraph 2.1 shall be used for carrying out official tuberculin skin test following the procedures referred to in paragraph 2.2.

2.1. **Standards for tuberculin (bovine and avian)**

2.1.1. *Definition*

Tuberculin purified protein derivative (tuberculin PPD, bovine or avian) is a preparation obtained from the heat-treated products of growth and lysis of *Mycobacterium bovis* or *Mycobacterium avium* (as appropiate) capable of revealing a delayed hypersensitivity in an animal sensitised to microorganisms of the same species.

2.1.2. *Production*

It is obtained from the water-soluble fractions prepared by heating in free-flowing steam and subsequently filtering cultures of *M. bovis* or *M. avium* (as appropiate) grown in a liquid synthetic medium. The active fraction of the filtrate, consisting mainly of protein, is isolated by precipitation, washed and re-dissolved. An antimicrobial preservative that does not give rise to false positive reactions, such as phenol, may be added. The final sterile preparation, free from mycobacteria, is distributed aseptically into sterile tamper-proof glass containers which are then closed so as to prevent contamination. The preparation may be freeze-dried.

2.1.3. *Identification of the product*

Inject a range of graded doses intradermally at different sites into suitably sensitised albino guinea-pigs, each weighing not less than 250 g. After 24 h to 28 h, reactions appear in the form of oedematous swellings with erythema with or without necrosis at the points of injection. The size and severity of the reactions vary according to the dose. Unsensitised guinea-pigs show no reactions to similar injections.

2.1.4. *Tests*

2.1.4.1. pH: The pH is 6.5 to 7.5.

2.1.4.2. Phenol: If the preparation to be examined contains phenol, its concentration is not more than 5 g/l.

2.1.4.3. Sensitising effect: Use a group of three guinea-pigs that have not been treated with any material which will interfere with the test. On 3 occasions at intervals of five days inject intradermally into each guinea-pig a dose of the preparation to be examined equivalent to 500 IU in 0,1 ml. 15 to 21 days after the third injection inject the same dose (500 IU) intradermally into these animals and into a control group of three guinea-pigs of the same mass and which have not previously received injections of tuberculin. 24 to 28 hours after the last injections, the reactions of the two groups are not significantly different.

2.1.4.4. Toxicity: Use two guinea-pigs, each weighing not less than 250 g and which have not previously been treated with any material which will interfere with the test. Inject subcutaneously into each guinea-pig 0,5 ml of the preparation to be examined. Observe the animals for seven days. No abnormal effects occur during the observation period.

2.1.4.5. Sterility: It complies with the test for sterility prescribed in the monograph on Vaccines for veterinary use 4th Edition 2002 of the European Pharmacopoeia.

2.1.5. *Potency*

The potency of tuberculin purified protein derivative (bovine and avian) is determined by comparing the reactions produced in sensitised guinea-pigs by the intradermal injection of a series of dilutions of the preparation to be examined with those produced by known concentrations of a reference preparation of tuberculin (bovine or avian, as appropiate) purified protein derivative calibrated in International Units.

To test the potency, sensitise not fewer than nine albino guinea-pigs, each weighing 400 g to 600 g, by the deep intramuscular injection of 0,0001 mg of wet mass of living *M. bovis* of strain AN5 suspended in 0.5 ml of a 9 g/l solution of sodium chloride R for bovine tuberculin, or a suitable dose of inactivated or live *M. avium* for avian tuberculin. Not less than four weeks after the sensitisation of the guinea-pigs, shave their flanks to provide space for not more than four injection sites on each side. Prepare dilutions of the preparation to be examined and of the reference preparation using isotonic phosphate-buffered saline (pH 6,5-7,5) containing 0,005 g/l of polysorbate 80 R. Use not fewer than three doses of the reference preparation and not fewer than three doses of the preparation to be examined. Choose the doses such that the lesions produced have a diameter of not less than 8 mm and not more than 25 mm. Allocate the dilutions randomly to the sites using a Latin square design. Inject each dose intradermally in a constant volume of 0,1 ml or 0,2 ml. Measure the diameters of the lesions after 24 to 28 hours and calculate the result of the test using the usual statistical methods and assuming that the diameters of the lesions are directly proportional to the logarithm of the concentration of the tuberculins.

The test is not valid unless the fiducial limits of error (P = 0,95) are not less than 50 % and not more then 200 % of the estimated potency. The estimated potency is not less than 66 % and not more than 150 % of the stated potency for bovine tuberculin. The estimated potency is not less than 75 % and not more than 133 % of the stated potency for avian tuberculin. The stated potency is not less than 20 000 IU/ml for both tuberculins (bovine and avian).

2.1.6. *Storage*

Store protected from light, at a temperature of 5 ± 3 °C.

2.1.7. *Labelling*

The label states:

— the potency in International Units per millilitre,

— the name and quantity of any added substance,

— for freeze-dried preparations:

  — -the name and volume of the reconstituting liquid to be added,

  — -that the product should be used immediately after reconstitution.

2.2. **Test procedures**

2.2.1. The following shall be recognised as official intradermal tuberculin tests:

— the single intradermal test: this test requires a single injection of bovine tuberculin,

— the intradermal comparative test: this test requires one injection of bovine tuberculin and one injection of avian tuberculin given simultaneously.