

[^{F1}][^{F2}ANNEX B

TUBERCULOSIS

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 1226/2002 of 8 July 2002 amending Annex B to Council Directive 64/432/EEC.

2. THE TUBERCULIN SKIN TEST

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 appropriate) 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 appropriate) 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.

Status: EU Directives are being published on this site to aid cross referencing from UK legislation. After IP completion day (31 December 2020 11pm) no further amendments will be applied to this version.

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 appropriate) 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 than 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.]]