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Bovine Tuberculin Purified Protein Derivative

General Notices

Bovine Tuberculin P.P.D.

(Ph. Eur. monograph 0536)

Ph Eur

DEFINITION

Bovine tuberculin purified protein derivative (bovine tuberculin PPD) is a preparation obtained from the heat-treated products of growth and lysis of *Mycobacterium bovis* capable of revealing a delayed hypersensitivity in an animal sensitised to micro-organisms of the same species.

PRODUCTION

It is obtained from the water-soluble fractions prepared by heating in free-flowing steam and subsequently filtering cultures of *M. bovis* 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-evident glass containers, which are then closed so as to prevent contamination. The preparation may be freeze-dried.

The identification, the tests and the determination of potency apply to the liquid form and to the freeze-dried form after reconstitution as stated on the label.

IDENTIFICATION

Inject a range of graded doses intradermally at different sites into suitably sensitised albino guinea-pigs, each weighing not less than 250 g. After 24-28 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.

TESTS

pH (*2.2.3*)

6.5 to 7.5.

Phenol (*2.5.15*)

Maximum 5 g/L, if the preparation to be examined contains phenol.

Sensitising effect

Use a group of 3 guinea-pigs that have not been treated with any material that will interfere with the test. On 3 occasions at intervals of 5 days, inject intradermally into each guinea-pig a dose of the preparation to be examined equivalent to 500 IU in 0.1 mL. 15-21 days after the 3rd injection, inject the same dose (500 IU) intradermally into these animals and into a control group of 3 guinea-pigs of the same mass, which have not previously received injections of tuberculin. 24-28 h after the last injections, the reactions of the 2 groups are not significantly different.

Toxicity

Use 2 guinea-pigs, each weighing not less than 250 g, that have not previously been treated with any material that will interfere with the test. Inject subcutaneously into each guinea-pig 0.5 mL of the preparation to be examined. Observe the animals for 7 days. No abnormal effects occur during the observation period.

Sterility

It complies with the test for sterility prescribed in the monograph *Vaccines for veterinary use (0062)*.

POTENCY

The potency of bovine tuberculin purified protein derivative 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 calibrated in International Units.

The International Unit is the activity contained in a stated amount of the International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organization.

Sensitise not fewer than 8 albino guinea-pigs, each weighing 400-600 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*. Not less than 4 weeks after the sensitisation of the guinea-pigs, shave their flanks to provide space for not more than 4 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 3 doses of the reference preparation and not fewer than 3 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, for example 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 24-28 h and calculate the results of the test using the usual statistical methods (for example, *5.3*) 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 confidence limits (P = 0.95) are not less than 50 per cent and not more than 200 per cent of the estimated potency. The estimated potency is not less than 66 per cent and not more than 150 per cent of the stated potency. The stated potency is not less than 20 000 IU/mL.

STORAGE

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

LABELLING

The label states:

— the potency in International Units per millilitre;

— the name and quantity of any excipient;

— for freeze-dried preparations:

— the name and volume of the reconstituting liquid to be added;

— that the product is to be used immediately after reconstitution.