ASSAY
The potency of old tuberculin is determined by comparing the reactions produced by the intradermal injection of increasing doses of the preparation to be examined into sensitised guinea-pigs with the reactions produced by known concentrations of the reference preparation.
Prepare a suspension containing a suitable amount (0.1 mg to 0.4 mg/ml) of heat-inactivated, dried mycobacteria in mineral oil with or without emulsifier; use mycobacteria of a strain of the same species as that used in the preparation to be examined. Sensitise not fewer than 6 pale-coloured guinea-pigs weighing not less than 300 g by injecting intramuscularly or intradermally a total of about 0.5 ml of the suspension, divided between several sites if necessary. Carry out the test after the period of time required for optimal sensitisation which is usually 4 to 8 weeks after sensitisation. Depilate the flanks of the animals so that it is possible to make at least three injections on each side and not more than a total of 12 injection points per animal. Use at least three different doses of the reference preparation and at least 3 different doses of the preparation to be examined. For both preparations, use doses such that the highest dose is about 10 times the lowest dose. Choose the doses such that when they are injected the lesions produced have a diameter of not less than 8 mm and not more than 25 mm. In any given test, the order of the dilutions injected at each point is chosen at random in 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 24 h to 48 h later and calculate the results of the test by the usual statistical methods, assuming that the diameters of the lesions are directly proportional to the logarithm of the concentration of the preparation.
The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 64 per cent and not more than 156 per cent of the stated potency.

STORAGE
Store protected from light.

LABELLING
The label states:
– the number of International Units per millilitre,
– the species of mycobacterium used to prepare the product,
– the name and quantity of any antimicrobial preservative or other substance added to the preparation,
– the expiry date,
– where applicable, that old tuberculin is not to be injected in its concentrated form but diluted so as to administer not more than 100 IU per dose.

01/2005:0535

TUBERCULIN PURIFIED PROTEIN DERIVATIVE, AVIAN

Tuberculini aviarii derivatum proteinosum purificatum

DEFINITION
Tuberculin purified protein derivative, avian (Tuberculin PPD, avian) is a preparation obtained from the heat-treated products of growth and lysis of Mycobacterium avium 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. avium grown in a liquid synthetic medium. The active fraction of the filtrate, consisting mainly of protein, is isolated by precipitation, washed and redissolved. 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. 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 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.

TESTS

pH (2.2.3). The pH is 6.5 to 7.5.

Phenol (2.5.15). If the preparation to be examined contains phenol, its concentration is not more than 5 g/l.

Sensitising effect. Use a group of three guinea-pigs that have not been treated with any material which will interfere with the test. On three 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. Fifteen to twenty-one 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 h to 28 h after the last injections, the reactions of the two groups are not significantly different.

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 7 days. No abnormal effects occur during the observation period.

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

POTENCY
The potency of tuberculin purified protein derivative, 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 purified protein derivative, avian 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 Organisation.
Sensitise not fewer than nine albino guinea-pigs, each weighing 400 g to 600 g, by the deep intramuscular injection of a suitable dose of inactivated or live M. avium. 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 to 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 h to 28 h 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 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 75 per cent and not more than 133 per cent of the stated potency. The stated potency is not less than 20 000 IU/ml.

STORAGE
Store 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 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.

01/2005:0536

TUBERCULIN PURIFIED PROTEIN DERIVATIVE, BOVINE

Tuberculin bovini derivatum proteinosum purificatum

DEFINITION
Tuberculin purified protein derivative, bovine (Tuberculin PPD, bovine) 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-proof 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 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.

TESTS

ph (2.2.3). The pH is 6.5 to 7.5.

Phenol (2.5.15). If the preparation to be examined contains phenol, its concentration is not more than 5 g/l.

Sensitising effect. Use a group of 3 guinea-pigs that have not been treated with any material which 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 to 21 days after the third injection inject the same dose (500 IU) intradermally into these animals and into a control group of 3 guinea-pigs of the same mass and which have not previously received injections of tuberculin. 24 h to 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 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 7 days. No abnormal effects occur during the observation period.

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

POTENCY
The potency of tuberculin purified protein derivative, bovine 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.

Sensitise not fewer than 9 albino guinea-pigs, each weighing 400 g to 600 g, by the deep intramuscular injection of 0.001 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 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 h to 28 h 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 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.