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Mallein Purified Protein Derivative

General Notices

Mallein P.P.D.

DEFINITION

Mallein Purified Protein Derivative is a preparation of the heat treated products of growth and lysis of *Pseudomonas mallei*. It contains not less than 0.95 mg per mL and not more than 1.05 mg per mL of purified protein derivative.

PRODUCTION

It is prepared from the water-soluble fractions obtained by heating in free-flowing steam and subsequently filtering cultures of the glanders bacillus grown in a liquid synthetic medium. The active fraction of the filtrate, which is predominantly protein, is isolated by precipitation, washed and redissolved in phosphate buffered saline at neutral pH. It is then distributed in sterile containers that are inert towards the contents and sealed so as to exclude micro-organisms. A suitable preservative may be added.

CAUTION *Mallein P.P.D. is not dangerous to man, but the organism from which it is prepared is pathogenic to man and may be fatal if an infection is untreated. If an infection is suspected treatment should begin without delay*.

IDENTIFICATION

Inject small doses intradermally into suitable guinea-pigs that have been sensitised with killed *P. mallei* in oily adjuvant. Oedematous swellings occur at the point of injection after 48 hours.

TESTS

Acidity or alkalinity

pH, 6.5 to 7.5, Appendix V L.

Phenol

For preparations containing phenol as a preservative, not more than 0.5% w/v, determined by the method described under Veterinary Antisera.

Sterility

Complies with the *test for sterility*, Appendix XVI A, using Method I: Membrane filtration, whenever possible and particularly when the volume in a container is greater than 100 mL, with the following modifications.

Incubate the media for not less than 14 days at 30° to 35° in the test intended to detect bacteria and at 20° to 25° in the test intended to detect fungi.

Use the quantities stated under Application of the test to injectable preparations except that when the quantity in each container[1] is 20 mL or more of a liquid, the minimum quantity to be used for each medium is 10% of the contents or 5 mL, whichever is the less.

ASSAY

To 2.5 mL of the substance being examined, add 2.5 mL of *water* and 2.5 mL of a 40% w/v solution of *trichloroacetic acid*, mix, allow to stand for 30 minutes and centrifuge for 15 minutes. Discard the supernatant liquid and dissolve the residue in 0.5 mL of 5M *sodium hydroxide*. Transfer the solution to a Kjeldahl flask with the aid of 6 mL of *water* and add about 0.1 g of a mixture of 100 parts of *potassium sulfate*, 10 parts of *copper (II) sulfate* and 5 parts of *selenium dioxide*, and 1 mL of *nitrogen-free sulfuric acid*. Evaporate the water and continue heating until a brown deposit appears. Dissolve the deposit by the addition of 0.5 mL of *hydrogen peroxide solution (100 vol)*, continue heating until white fumes of sulfur trioxide appear and boil rapidly for at least 10 minutes. (If while heating a brown deposit again appears, add a further 0.5 mL of *hydrogen peroxide solution (100 vol)*. Transfer to an ammonia distillation apparatus with the aid of 5 mL of *water* and add 5 mL of a 50% w/v solution of *sodium hydroxide* to form a lower layer. Distil for 3 minutes, collecting the distillate in a mixture of 5 mL of a 2% w/v solution of *boric acid* and 0.05 mL of a solution containing 0.066% w/v of *methyl red* and 0.033% w/v of *bromocresol green* in *ethanol (96%)* and titrate with *0.00447M sulfuric acid VS* (prepared by diluting 89.3 mL of *0.05M sulfuric acid VS* to 1000 mL with *water*). Repeat the operation using 2.5 mL of *water* in place of the preparation being examined. The difference between the titrations represents the ammonia liberated by the substance being tested. Each mL of *0.00447M sulfuric acid VS* is equivalent to 0.875 mg of purified protein derivative.

STORAGE

Mallein Purified Protein Derivative should be protected from light and stored at a temperature between 2° and 8°. Under these conditions it may be expected to retain its potency for not less than 6 months.

LABELLING

The label states (1) the volume of the contents; (2) the date after which the preparation is not intended to be used; (3) that the preparation is to be used for animals only; (4) the conditions under which it should be stored; (5) the name and percentage of any added preservative; (6) the dose.

ANNEX

Guidance to manufacturers performing the test for sterility. In determining the number of containers to be tested, the manufacturer should have regard to the environmental conditions of manufacture, the volume of preparation per container and any other special considerations applying to the preparation concerned. With respect to diagnostic preparations for veterinary use, 1% of the containers in a batch, with a minimum of three and a maximum of 10 is considered a suitable number assuming that the preparation has been manufactured under appropriately validated conditions designed to exclude contamination.

1 Guidance to manufacturers on the number of containers is provided in the Annex to this monograph.