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BOTULINUM ANTITOXIN

Immunoserum botulinicum

DEFINITION

Botulinum antitoxin is a preparation containing antitoxic globulins that have the power of specifically neutralising the toxins formed by *Clostridium botulinum* type A, type B or type E, or any mixture of these types.

PRODUCTION

It is obtained by fractionation from the serum of horses, or other mammals, that have been immunised against *Cl. botulinum* type A, type B and type E toxins.

IDENTIFICATION

It specifically neutralises the types of *Cl. botulinum* toxins stated on the label, rendering them harmless to susceptible animals.

POTENCY

Not less than 500 IU of antitoxin per millilitre for each of types A and B and not less than 50 IU of antitoxin per millilitre for type E.

The potency of botulinum antitoxin is determined by comparing the dose necessary to protect mice against the lethal effects of a fixed dose of botulinum toxin with the quantity of the standard preparation of botulinum antitoxin necessary to give the same protection. For this comparison a reference preparation of each type of botulinum antitoxin, calibrated in International Units, and suitable preparations of botulinum toxins, for use as test toxins, are required. The potency of each test toxin is determined in relation to the specific reference preparation; the potency of the botulinum antitoxin to be examined is determined in relation to the potency of the test toxins by the same method.

International Units of the antitoxin are the specific neutralising activity for botulinum toxin type A, type B and type E contained in stated amounts of the International Standards which consist of dried immune horse sera of types A, B and E. The equivalence in International Units of the International Standard is stated from time to time by the World Health Organisation.

Selection of animals. Use mice having body masses such that the difference between the lightest and the heaviest does not exceed 5 g.

Preparation of test toxins. CAUTION: Botulinum toxin is extremely toxic: exceptional care must be taken in any procedure in which it is employed. Prepare type A, B and E toxins from sterile filtrates of approximately 7-day cultures in liquid medium of *Cl. botulinum* types A, B and E. To the filtrates, add 2 volumes of glycerol, concentrate, if necessary, by dialysis against glycerol and store at or slightly below 0 °C.

Selection of test toxins. Select toxins of each type for use as test toxins by determining for mice the L+/10 dose and the LD_{50}, the observation period being 96 h. The test toxins contain at least 1000 LD_{50} in an L+/10 dose.

Determination of test doses of the toxins (L+/10 dose). Prepare solutions of the reference preparations in a suitable liquid such that each contains 0.25 IU of antitoxin per millilitre. Using each solution in turn, determine the test dose of the corresponding test toxin.

Prepare mixtures of the solution of the reference preparation and the test toxin such that each contains 2.0 ml of the solution of the reference preparation, one of a graded series of volumes of the test toxin and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 60 min. Using four mice for each mixture, inject a dose of 1.0 ml intraperitoneally into each mouse. Observe the mice for 96 h.

The test dose of toxin is the quantity in 1.0 ml of the mixture made with the smallest amount of toxin capable of causing, despite partial neutralisation by the reference preparation, the death of all four mice injected with the mixture within the observation period.

Determination of potency of the antitoxin. Prepare solutions of each reference preparation in a suitable liquid such that each contains 0.25 IU of antitoxin per millilitre.

Prepare solutions of each test toxin in a suitable liquid such that each contains 2.5 test doses per millilitre.

Using each toxin solution and the corresponding reference preparation in turn, determine the potency of the antitoxin. Prepare mixtures of the solution of the test toxin and the antitoxin to be examined such that each contains 2.0 ml of the solution of the test toxin, one of a graded series of volumes of the antitoxin to be examined, and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Also prepare mixtures of the solution of the test toxin and the solution of the reference preparation such that each contains 2.0 ml of the solution of the test toxin, one of a graded series of volumes of the solution of the reference preparation centred on that volume (2.0 ml) that contains 0.5 IU, and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 60 min. Using four mice for each mixture, inject a dose of 1.0 ml intraperitoneally into each mouse. Observe the mice for 96 h.

The mixture that contains the largest volume of antitoxin that fails to protect the mice from death contains 0.5 IU. This quantity is used to calculate the potency of the antitoxin in International Units per millilitre.

The test is not valid unless all the mice injected with mixtures containing 2.0 ml or less of the solution of the reference preparation die and all those injected with mixtures containing more survive.

LABELLING

The label states the types of *Cl. botulinum* toxin neutralised by the preparation.

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DIPHTHERIA ANTITOXIN

Immunoserum diphthericum

DEFINITION

Diphtheria antitoxin is a preparation containing antitoxic globulins that have the power of specifically neutralising the toxin formed by *Corynebacterium diphtheriae*.

PRODUCTION

It is obtained by fractionation from the serum of horses, or other mammals, that have been immunised against diphtheria toxin.

IDENTIFICATION

It specifically neutralises the toxin formed by *C. diphtheriae*, rendering it harmless to susceptible animals.