CLOSTRIDIUM NOVYI ALPHA ANTITOXIN FOR VETERINARY USE

Immunoserum clostridii novyi alpha ad usum veterinarium

DEFINITION
Clostridium novyi alpha antitoxin for veterinary use is a preparation containing the globulins that have the power of specifically neutralising the alpha toxin formed by Clostridium novyi (Former nomenclature: Clostridium oedematiosi). It consists of the serum or a preparation obtained from the serum of animals immunised against Cl. novyi alpha toxin.

IDENTIFICATION
It specifically neutralises the alpha toxin of Cl. novyi rendering it harmless to susceptible animals.

POTENCY
The potency of the crude serum is not less than 750 IU/ml when obtained from horses and not less than 250 IU/ml when obtained from cattle.

The potency of the concentrated serum is not less than 1500 IU/ml when obtained from horses and not less than 500 IU/ml when obtained from cattle.

The International Unit is the specific neutralising activity for Cl. novyi alpha toxin contained in a stated amount of the International Standard, which consists of a quantity of dried immune horse serum. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

The potency of clostridium novyi alpha antitoxin is determined by comparing the dose necessary to protect mice or other suitable animals against the toxic effects of a fixed dose of Cl. novyi alpha toxin with the quantity of a reference preparation of clostridium novyi alpha antitoxin, calibrated in International Units, necessary to give the same protection. For this comparison, a suitable preparation of Cl. novyi alpha toxin for use as a test toxin is required.

The dose of the test toxin is determined in relation to the reference preparation; the potency of the clostridium novyi alpha antitoxin to be examined is determined in relation to the reference preparation using the test toxin.

Preparation of test toxin. Prepare the test toxin from a sterile filtrate of an approximately 5-day culture in liquid medium of Cl. novyi type B and dry by a suitable method. Select the toxin by determining for mice the L+/10 dose and the LD₅₀, the observation period being 72 h. A suitable alpha toxin contains not less than one L+/10 dose in 0.05 mg and not less than 10 LD₅₀ in each L+/10 dose.

Determination of test dose of toxin. Prepare a solution of the reference preparation in a suitable liquid so that it contains 1 IU/ml. Prepare a solution of the test toxin in a suitable liquid so that 1 ml contains a precisely known amount such as 1 mg. Prepare mixtures of the solution of the reference preparation and the solution of the test toxin such that each mixture contains 1.0 ml of the solution of the reference preparation (1 IU), one of a series of graded volumes of the solution of the test toxin and sufficient of a suitable liquid to bring the total volume to 2.0 ml. Allow the mixtures to stand at room temperature for 60 min. Using not fewer than two mice, for each mixture, inject a dose of 0.2 ml intramuscularly or subcutaneously into each mouse. Observe the mice for 72 h.

If all the mice die, the amount of toxin present in 0.2 ml of the mixture is in excess of the test dose. If none of the mice dies, the amount of toxin present in 0.2 ml of the mixture is less than the test dose. Prepare similar fresh mixtures such that 2.0 ml of each mixture contains 1.0 ml of the reference preparation (1 IU) and one of a series of graded volumes of the solution of the test toxin separated from each other by steps of not more than 20 per cent and covering the expected end-point. Allow the mixtures to stand at room temperature for 60 min.

Using not fewer than two mice for each mixture, inject a dose of 0.2 ml intramuscularly or subcutaneously into each mouse. Observe the mice for 72 h. Repeat the determination at least once and combine the results of the separate tests that have been made with mixtures of the same composition so that a series of totals is obtained, each total representing the mortality due to a mixture of a given composition. The test dose of toxin is the amount present in 0.2 ml of that mixture which causes the death of one half of the total number of mice injected with it.

Determination of the potency of the antitoxin to be examined

Preliminary test. Dissolve a quantity of the test toxin in a suitable liquid so that 1 ml contains ten times the test dose. Prepare mixtures of the solution of the test toxin and of the antitoxin to be examined such that each mixture contains 1.0 ml of the solution of the test toxin, one of a series of graded volumes of the antitoxin to be examined and sufficient of a suitable liquid to bring the final volume to 2.0 ml. Allow the mixtures to stand at room temperature for 60 min. Using not fewer than two mice for each mixture, inject a dose of 0.2 ml intramuscularly or subcutaneously into each mouse. Observe the mice for 72 h. If none of the mice dies, the amount of the mixture contains more than 0.1 IU. If all the mice die, 0.2 ml of the mixture contains less than 0.1 IU.

Final test. Prepare mixtures of the solution of the test toxin and of the antitoxin to be examined such that 2.0 ml of each mixture contains 1.0 ml of the solution of the test toxin and one of a series of graded volumes of the antitoxin to be examined, separated from each other by steps of not more than 20 per cent and covering the expected end-point as determined by the preliminary test. Prepare further mixtures such that 2.0 ml of each mixture contains 1.0 ml of the solution of the test toxin and one of a series of graded volumes of the solution of the reference preparation, in order to confirm the test dose of the toxin. Allow the mixtures to stand at room temperature for 60 min. Using not fewer than two mice for each mixture, proceed as described in the preliminary test. The test mixture which contains 0.1 IU in 0.2 ml is that mixture which kills the same or almost the same number of mice as the reference mixture containing 0.1 IU in 0.2 ml. Repeat the determination at least once and calculate the average of all valid estimates. Estimates are valid only if the reference preparation gives a result within 20 per cent of the expected value.

The confidence limits (P = 0.95) have been estimated to be: 85 per cent and 114 per cent when two animals per dose are used, 91.5 per cent and 109 per cent when four animals per dose are used, 93 per cent and 108 per cent when six animals per dose are used.

LABELLING
The label states whether the product consists of crude or concentrated serum.