ASSAY
Not less than 1000 IU of antitoxin per millilitre for antitoxin obtained from horse serum. Not less than 500 IU of antitoxin per millilitre for antitoxin obtained from the serum of other mammals.

The potency of diphtheria antitoxin is determined by comparing the dose necessary to protect guinea-pigs or rabbits against the erythrogenic effects of a fixed dose of diphtheria toxin with the quantity of the standard preparation of diphtheria antitoxin necessary to give the same protection. For this comparison a reference preparation of diphtheria antitoxin, calibrated in International Units, and a suitable preparation of diphtheria toxin, for use as a test toxin, are required. The potency of the test toxin is determined in relation to the reference preparation; the potency of the diphtheria antitoxin to be examined is determined in relation to the potency of the test toxin by the same method.

The International Unit of antitoxin is the specific neutralising activity for diphtheria 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.

Preparation of test toxin. Prepare diphtheria toxin from cultures of C. diphtheriae in a liquid medium. Filter the culture to obtain a sterile toxic filtrate and store at 4 °C

Selection of test toxin. Select a toxin for use as a test toxin by determining for guinea-pigs or rabbits the lr/100 dose and the minimal reacting dose, the observation period being 48 h. The test toxin has at least 200 minimal reacting doses in the lr/100 dose.

Minimal reacting dose. This is the smallest quantity of toxin, which, when injected intracutaneously into guinea-pigs or rabbits, causes a small, characteristic reaction at the site of injection within 48 h.

The test toxin is allowed to stand for some months before being used for the assay of antitoxin. During this time its toxicity declines and the lr/100 dose may be increased. Determine the minimal reacting dose and the lr/100 dose at frequent intervals. When experiment shows that the lr/100 dose is constant, the test toxin is ready for use and may be used for a long period. Store the test toxin in the dark at 0 °C to 5 °C. Maintain its sterility by the addition of toluene or other antimicrobial preservative that does not cause a rapid decline in specific toxicity.

Determination of test dose of toxin (lr/100 dose). Prepare a solution of the reference preparation in a suitable liquid such that it contains 0.1 IU of antitoxin per millilitre.

Prepare mixtures of the solution of the reference preparation and of the test toxin such that each contains 1.0 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 2.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 15 min to 60 min. Using two animals for each mixture, inject a dose of 0.2 ml intracutaneously into the shaven or depilated flanks of each animal. Observe the animals for 48 h.

The test dose of toxin is the quantity in 0.2 ml of the mixture made with the smallest amount of toxin capable of causing, despite partial neutralisation by the reference preparation, a small but characteristic erythematous lesion at the site of injection.

Determination of potency of the antitoxin. Prepare a solution of the reference preparation in a suitable liquid such that it contains 0.125 IU of antitoxin per millilitre. Prepare a solution of the test toxin in a suitable liquid such that it contains 12.5 test doses per millilitre.

Prepare mixtures of the solution of the test toxin and of the antitoxin to be examined such that each contains 0.8 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 2.0 ml. Also prepare mixtures of the solution of the test toxin and the solution of the reference preparation such that each contains 0.8 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 (0.8 ml) that contains 0.1 IU and sufficient of a suitable liquid to bring the total volume to 2.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 15 min to 60 min. Using two animals for each mixture, inject a dose of 0.2 ml intracutaneously into the shaven or depilated flanks of each animal. Observe the animals for 48 h.

The mixture that contains the largest volume of antitoxin that fails to protect the guinea-pigs from the erythematous effects of the toxin contains 0.1 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 sites injected with mixtures containing 0.8 ml or less of the solution of the reference preparation show erythematous lesions and at all those injected with mixtures containing more there are no lesions.

01/2005:0090

GAS-GANGRENE ANTITOXIN, MIXED
Immunoserum gangraenicum mixtum

DEFINITION
Mixed gas-gangrene antitoxin is prepared by mixing gas-gangrene antitoxin (novyi), gas-gangrene antitoxin (perfringens) and gas-gangrene antitoxin (septicum) in appropriate quantities.

IDENTIFICATION
It specifically neutralises the alpha toxins formed by Clostridium novyi (former nomenclature: Clostridium oedematis), Clostridium perfringens and Clostridium septicum, rendering them harmless to susceptible animals.

ASSAY
Gas-gangrene antitoxin (novyi), not less than 1000 IU of antitoxin per millilitre; gas-gangrene antitoxin (perfringens), not less than 1000 IU of antitoxin per millilitre; gas-gangrene antitoxin (septicum) not less than 500 IU of antitoxin per millilitre.

Carry out the assay for each component, as prescribed in the monographs on Gas-gangrene antitoxin (novyi) (0087), Gas-gangrene antitoxin (perfringens) (0088) and Gas-gangrene antitoxin (septicum) (0089).

01/2005:0087

GAS-GANGRENE ANTITOXIN (NOVYI)
Immunoserum gangraenicum
(Clostridium novyi)

DEFINITION
Gas-gangrene antitoxin (novyi) is a preparation containing antitoxic globulins that have the power of neutralising the alpha toxin formed by Clostridium novyi (Former...