2.7.6. Assy of diphtheria vaccine (adsorbed)

The potency of diphtheria vaccine (adsorbed) is determined by comparing the dose of the vaccine required to protect guinea-pigs from the effects of either an erythrogenic dose of diphtheria toxin administered intradermally or a lethal dose of diphtheria toxin administered subcutaneously with the dose of a reference preparation, calibrated in International Units, needed to give the same protection.

The International Unit is the activity contained in a stated amount of the International Standard which consists of a quantity of diphtheria toxoid adsorbed on aluminium hydroxide. The equivalence in International Units of the International Standard is stated by the World Health Organization.

Diphtheria vaccine (adsorbed) BRP is suitable for use as a reference preparation.

The design of the assay described below follows a parallel-line model with 3 dilutions for the test and reference preparations. Once the analyst has sufficient experience with this method for a given vaccine, it is possible to apply a simplified model using a single dilution for both test and reference preparations. Such a model enables the analyst to determine whether the potency of the test preparation is significantly higher than the minimum required but does not give information on linearity, parallelism and the dose-response curve. The simplified model leads to a considerable reduction in the number of experimental animals required and must be considered by each analyst in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

METHOD OF INTRADERMAL CHALLENGE

Selection and distribution of the test animals. Use in the test, healthy, white guinea-pigs from the same stock and of a size suitable for the prescribed number of challenge sites, the difference in body mass between the heaviest and the lightest animal being not greater than 100 g. Distribute the guinea-pigs in not fewer than 6 equal groups; use groups containing a number of animals sufficient to obtain results that fulfil the requirements for a valid assay prescribed below. If the challenge toxin to be used has not been shown to be stable or has not been adequately standardised, include 5 guinea-pigs as unvaccinated controls. Use guinea-pigs of the same sex or with males and females equally distributed between the groups.

Selection of the challenge toxin. Select a preparation of diphtheria toxin containing 67 to 153 Lf/100 in 1 Lf and 25 000 to 50 000 minimal reacting doses for guinea-pig skin in 1 Lf. If the challenge toxin preparation has been shown to be stable, it is not necessary to verify the activity for every assay.

Preparation of the challenge toxin solution. Immediately before use, dilute the challenge toxin with a suitable diluent to obtain a challenge toxin solution containing about 0.0512 Lf in 0.2 ml. Prepare from this a further series of 5 four-fold dilutions containing about 0.0128, 0.0032, 0.0008, 0.0002 and 0.00005 Lf in 0.2 ml.

Determination of potency of the vaccine. Using a 9 g/l solution of sodium chloric chloride R, prepare dilutions of the vaccine to be examined and of the reference preparation, such that for each, the dilutions form a series differing by not more than 2.5-fold steps and in which the intermediate dilutions, when injected subcutaneously at a dose of 1.0 ml per guinea-pig, will result in an intradermal score of approximately 3 when the animals are challenged. Allocate the dilutions 1 to each of the groups of guinea-pigs and inject subcutaneously 1.0 ml of each dilution into each guinea-pig in the group to which that dilution is allocated. After 28 days, shave both flanks of each guinea-pig and inject 0.2 ml of each of the 6 toxin dilutions intradermally into 6 separate sites on each of the vaccinated guinea-pigs in such a way as to minimise interference between adjacent sites.

Determination of the activity of the challenge toxin. If necessary, inject the unvaccinated control animals with dilutions containing 80, 40, 20, 10 and 5 millionths of an Lf of the challenge toxin.

Reading and interpretation of results. Examine all injection sites 48 h after injection of the challenge toxin and record the incidence of specific diphtheria erythema. Record also the number of sites free from such reactions as the intra-dermal challenge score. Tabulate together the intradermal challenge scores for all the animals receiving the same dilution of vaccine and use those data with a suitable transformation, such as (score)$^2$ or arcsin((score/6)$^2$), to obtain an estimate of the relative potency for each of the test preparations by parallel-line quantitative analysis.

Requirements for a valid assay. The test is not valid unless:

- for both the vaccine to be examined and the reference preparation, the mean score obtained at the lowest dose level is less than 3 and the mean score at the highest dose level is more than 3,
- if applicable, the toxin dilution that contains 40 millionths of an Lf gives a positive erythema in at least 80 per cent of the control guinea-pigs and the dilution containing 20 millionths of an Lf gives a positive erythema in less than 80 per cent of the guinea-pigs (if these criteria are not met a different toxin has to be selected),
- 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 statistical analysis shows no deviation from linearity and parallelism.

The test may be repeated but when more than 1 test is performed the results of all valid tests must be combined in the estimate of potency.
METHOD OF LETHAL CHALLENGE

Selection and distribution of the test animals. Use in the test healthy guinea-pigs from the same stock, each weighing 250 g to 350 g. Distribute the guinea-pigs in not fewer than 6 equal groups; use groups containing a number of animals sufficient to obtain results that fulfil the requirements for a valid assay prescribed below. If the challenge toxin to be used has not been shown to be stable or has not been adequately standardised, include 4 further groups of 5 guinea-pigs as unvaccinated controls. Use guinea-pigs of the same sex or with males and females equally distributed between the groups.

Selection of the challenge toxin. Select a preparation of diphtheria toxin containing not less than 100 LD₅₀ per millilitre. If the challenge toxin preparation has been shown to be stable, it is not necessary to verify the lethal dose for every assay.

Preparation of the challenge toxin solution. Immediately before use, dilute the challenge toxin with a suitable diluent to obtain a challenge toxin solution containing approximately 100 LD₅₀ per millilitre. If necessary, dilute portions of the challenge toxin solution 1 in 32, 1 in 100 and 1 in 320 with the same diluent.

Determination of potency of the vaccine. Using a 9 g/l solution of sodium chloride R, prepare dilutions of the vaccine of the reference preparation, such that for each, the dilutions form a series differing by not more than 2.5-fold steps and in which the intermediate dilutions, when injected subcutaneously at a dose of 1.0 ml per guinea-pig, protect approximately 50 per cent of the animals from the lethal effects of the subcutaneous injection of the quantity of diphtheria toxin prescribed for this test. Allocate the dilutions 1 to each of the groups of guinea-pigs and inject subcutaneously 1.0 ml of each dilution into each guinea-pig in the group to which that dilution is allocated. After 28 days, inject subcutaneously into each animal 1.0 ml of the challenge toxin solution (100 LD₅₀). If the dilution may be expected to protect about 50 per cent of the survivors 4 days after injection of the challenge toxin solution into each guinea-pig, protect approximately 50 per cent of the guinea-pigs.

Determination of the activity of the challenge toxin. If necessary, allocate the challenge toxin solution and the 3 dilutions made from it, 1 to each of the 4 groups of 5 guinea-pigs and inject subcutaneously 1.0 ml of each solution into each guinea-pig in the group to which that solution is allocated.

Reading and interpretation of results. Count the number of surviving guinea-pigs 4 days after injection of the challenge toxin. Calculate the potency of the vaccine to be examined relative to the potency of the reference preparation on the basis of the proportion of animals surviving in each of the groups of vaccinated guinea-pigs, using the usual statistical methods.

Requirements for a valid assay. The test is not valid unless:

- the statistical analysis shows no deviation from linearity and parallelism.
- the test may be repeated but when more than 1 test is performed the results of all valid tests must be combined in the estimate of potency.

The potency of pertussis vaccine is determined by comparing the dose necessary to protect mice against the effects of a lethal dose of Bordetella pertussis, administered intracerebrally, with the quantity of a reference preparation, calibrated in International Units, needed to give the same protection. The International Unit is the activity contained in a stated amount of the International Standard which consists of a quantity of dried pertussis vaccine. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Selection and distribution of the test animals. Use in the test, healthy mice less than 5 weeks old of a suitable strain from the same stock, the difference in mass between the heaviest and the lightest being not greater than 5 g. Distribute the mice in 6 groups of not fewer than 16 and 4 groups of 10. The mice must all be of the same sex or the males and females should be distributed equally between the groups.

Selection of the challenge strain and preparation of the challenge suspension. Select a suitable strain of B. pertussis capable of causing the death of mice within 14 days of intracerebral injection. If more than 20 per cent of the mice die within 48 h of the injection the strain is not suitable. Make one subculture from the strain and suspend the harvested B. pertussis in a solution containing 10 g/l of casein hydrolysate R and 6 g/l of sodium chloride R and having a pH of 7.0 to 7.2 or in another suitable solution. Determine the opacity of the suspension. Prepare a series of dilutions in the same solution and allocate each dilution to a group of ten mice.Inject intracerebrally into each mouse a dose (0.02 ml or 0.03 ml) of the dilution allocated to its group. After 14 days, count the number of mice surviving in each group. From the results, calculate the expected opacity of a suspension containing 100 LD₅₀ in each challenge dose. For the test of the vaccine to be examined make a fresh subculture from the same strain of B. pertussis and prepare a suspension of the harvested organisms with an opacity corresponding to about 100 LD₅₀ in each challenge dose. Prepare 3 dilutions of the challenge suspension.

Determination of potency. Prepare 3 serial dilutions of the vaccine to be examined and 3 similar dilutions of the reference preparation such that in each the intermediate dilution may be expected to protect about 50 per cent of the mice from the lethal effects of the challenge dose of B. pertussis. Suggested doses are 1/8, 1/40 and 1/200 of the human dose of the vaccine to be examined and 0.5 IU, 0.1 IU and 0.02 IU of the reference preparation, each dose being contained in a volume not exceeding 0.5 ml. Allocate 6 dilutions one to each of the groups of not fewer than 16 mice and inject intraperitoneally into each mouse one dose of the dilution allocated to its group. After 14 to 17 days inject intracerebrally into each animal in the groups of not fewer than 16, one dose of the challenge suspension. Allocate the challenge suspension and the 3 dilutions made from it one to each of the groups of 10 mice and inject intracerebrally one dose of each suspension into each mouse in the group to which that suspension is allocated.