**DIPHTHERIA, TETANUS, PERTUSSIS AND POLIOMYELITIS (INACTIVATED) VACCINE ( ADSORBED)**

**DEFINITION**

Diphtheria, tetanus, pertussis and poliomyelitis (inactivated) vaccine (adsorbed) is a combined vaccine containing:

- inactivated suspension of *Corynebacterium diphtheriae*; 
- tetanus formol toxoid; 
- an inactivated suspension of *Bordetella pertussis*; 
- suitable strains of human polioviruses 1, 2 and 3 grown in suitable cell cultures and inactivated by a validated method; 
- a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

**PRODUCTION**

**GENERAL PROVISIONS**

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

*Reference vaccine(s).* Provided valid assays can be performed, monocOMPonent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocOMPonent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

**Specific toxicity of the diphtheria and tetanus components.**

The production method is validated to demonstrate that the product, if tested, would comply with the following tests: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing 250-350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than one animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

**PRODUCTION OF THE COMPONENTS**

The production of the components complies with the requirements of the monographs on *Diphtheria* vaccine (adsorbed) (0443), *Tetanus* vaccine (adsorbed) (0452), *Pertussis* vaccine (adsorbed) (0161) and *Poliomyelitis* vaccine (inactivated) (0214).

**FINAL BULK VACCINE**

The final bulk vaccine is prepared by adsorption onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate, separately or together, of suitable quantities of bulk purified diphtheria toxoid and bulk purified tetanus toxoid and admixture of suitable quantities of an inactivated suspension of *B. pertussis* and purified monovalent harvests of human polioviruses 1, 2 and 3 or a suitable quantity of a trivalent pool of such purified monovalent harvests. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

- **Bovine serum albumin.** Determined on the poliomyelitis components by a suitable immunochemical method (2.7.1) during preparation of the final bulk vaccine, before addition of the adsorbent, the amount of bovine serum albumin is such that the content in the final vaccine will be not more than 50 ng per single human dose.

- **Antimicrobial preservative.** Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the intended content.

- **Sterility (2.6.1).** Carry out the test for sterility using 10 ml for each medium.

**FINAL LOT**

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided that the tests for specific toxicity of the pertussis component and antimicrobial preservative, and the assays for the diphtheria, tetanus and pertussis components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided that the free formaldehyde content has been determined on the bulk purified antigens, the inactivated *B. pertussis* suspension and the purified monovalent harvests or the trivalent pool of polioviruses or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Provided that the in vivo assay for the poliomyelitis component has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

**Osmolality (2.2.35).** The osmolality of the vaccine is within the limits approved for the particular preparation.

**IDENTIFICATION**

A. Diphtheria toxoid is identified by a suitable immunochemical method (2.7.1). The following method, applicable to certain vaccines, is given as an example.

Dissolve in the vaccine to be examined sufficient *sodium citrate R* to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method (2.7.1). The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained during identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The centrifugation residue obtained in identification A may be used. Other suitable methods for separating the bacteria from the adsorbent may also be used. Identify pertussis vaccine by agglutination of the bacteria from the resuspended precipitate by antisera specific to *B. pertussis* or by the assay of the pertussis component prescribed under Assay.

D. The vaccine is shown to contain human polioviruses 1, 2 and 3 by a suitable immunochemical method (2.7.1) such as the determination of D-antigen by enzyme-linked immunosorbent assay (ELISA).
**TESTS**

**Specific toxicity of the pertussis component.** Use not fewer than 5 healthy mice each weighing 14-16 g for the vaccine group and for the saline control. Use mice of the same sex or distribute males and females equally between the groups. Allow the animals access to food and water for at least 2 h before injection and during the test. Inject each mouse of the vaccine group intraperitoneally with 0.5 ml containing a quantity of the vaccine equivalent to not less than half the single human dose. Inject each mouse of the control group with 0.5 ml of a 9 g/l sterile solution of sodium chloride R, preferably containing the same amount of antimicrobial preservative as that injected with the vaccine. Weigh the groups of mice immediately before the injection and 72 h and 7 days after the injection. The vaccine complies with the test if: (a) at the end of 72 h the total mass of the group of vaccinated mice is not less than that preceding the injection; (b) at the end of 7 days the average increase in mass per vaccinated mouse is not less than that preceding the injection; (c) not more than 5 per cent of the vaccinated mice die during the test. The test may be repeated and the results of the tests combined.

**Aluminium (2.5.13):** maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

**Free formaldeyde (2.4.18):** maximum 0.2 g/l.

**Antimicrobial preservative.** Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

**Sterility (2.6.1).** It complies with the test for sterility.

**ASSAY**

**Diphtheria component.** Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) (2.7.6).

The lower confidence limit \( P = 0.95 \) of the estimated potency is not less than 30 IU per single human dose.

**Tetanus component.** Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) (2.7.8).

If the test is carried out in guinea pigs, the lower confidence limit \( P = 0.95 \) of the estimated potency is not less than 40 IU per single human dose; if the test is carried out in mice, the lower confidence limit \( P = 0.95 \) of the estimated potency is not less than 60 IU per single human dose.

**Pertussis component.** Carry out the assay of pertussis vaccine (2.7.7).

The estimated potency is not less than 4 IU per single human dose and the lower confidence limit \( P = 0.95 \) of the estimated potency is not less than 2 IU per single human dose.

**Poliomyelitis component**

**D-antigen content.** As a measure of consistency of production, determine the D-antigen content for human polioviruses 1, 2 and 3 by a suitable immunochemical method (2.7.1) using a reference preparation calibrated in European Pharmacopoeia Units of D-antigen. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product. **Poliomyelitis vaccine (inactivated) BRP** is calibrated in European Pharmacopoeia Units and intended for use in the assay of D-antigen. The European Pharmacopoeia Unit and the International Unit are equivalent.

In *vivo* test. The vaccine complies with the in *vivo* assay of poliomyelitis vaccine (inactivated) (2.7.20).

**LABELLING**

The label states:

- the minimum number of International Units of diphtheria and tetanus toxoid per single human dose,
- the minimum number of International Units of pertussis vaccine per single human dose,
- the nominal amount of poliovirus of each type (1, 2 and 3), expressed in European Pharmacopoeia Units of D-antigen, per single human dose,
- the type of cells used for production of the poliomyelitis component,
- where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults,
- the name and the amount of the adsorbent,
- that the vaccine must be shaken before use,
- that the vaccine is not to be frozen.

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**DIPHTHERIA, TETANUS, PERTUSSIS, POLIOMYELITIS (INACTIVATED) AND HAEMOPHILUS TYPE b CONJUGATE VACCINE (ADSORBED)**

Vaccinum diphereriae, tetani, pertussis, poliomyelitidis inactivatum et haemophili stirpe b coniugatum adsorbatum

**DEFINITION**

Diphtheria, tetanus, pertussis, poliomyelitis (inactivated) and haemophilus type b conjugate vaccine (adsorbed) is a combined vaccine composed of: diphtheria formol toxoid; tetanus formol toxoid; an inactivated suspension of Bordetella pertussis; suitable strains of human polioviruses 1, 2 and 3 grown in suitable cell cultures and inactivated by a suitable method; polyribosylribitol phosphate (PRP) covalently bound to a carrier protein; a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate. The product is presented with the haemophilus component in a separate container, the contents of which are mixed with the other components immediately before use.

The formol toxoids are prepared from the toxins produced by the growth of Corynebacterium diphereriae and Clotridium tetani respectively.

PRP is a linear copolymer composed of repeated units of 3-β-D-ribofuranosyl(1→1)-ribofuranosyl-5-phosphate \([\text{C}_{10}H_{19}O_{12}P]_n\), with a defined molecular size and derived from a suitable strain of Haemophilus influenzae type b. The carrier protein, when conjugated to PRP, is capable of inducing a T-cell-dependent B-cell immune response to the polysaccharide.

**PRODUCTION**

**GENERAL PROVISIONS**

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.