PRP is determined either by assay of ribose (2.5.31) or phosphorus (2.5.18), by an immunochemical method (2.7.1) or by anion-exchange liquid chromatography (2.2.29) with pulsed-amperometric detection.

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

Free formaldehyde (2.4.18): maximum 0.2 g/l of free formaldehyde per single human dose.

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.

Water (2.5.12): maximum 3.0 per cent for the freeze-dried haemophilus component.

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 the minimum potency stated on the label unless otherwise justified and authorised, the minimum potency stated on the label is 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).

The lower confidence limit ($P = 0.95$) of the estimated potency is not less than 40 IU per single human dose.

Pertussis component. The vaccine complies with the assay of pertussis vaccine (acellular) (2.7.16).

Hepatitis B component. The vaccine complies with the assay of hepatitis B vaccine (2.7.15).

Polioimmunisation 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 D-antigen units. For each type, the amount of D-antigen per single human dose, the type and nominal amount of carrier protein per single human dose, 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, where applicable, that the vaccine contains a pertussis toxoid-like protein produced by genetic modification.

Vaccinum diptheriae, tetani, pertussis sine cellulis ex elementis praeparatum poliomyelitidis inactivatum et haemophilus stirpe b coniugatum adsorbatum

DEFINITION

Diphtheria, tetanus, pertussis (acellular, component), poliomyelitis (inactivated) and haemophilus type b conjugate vaccine (adsorbed) is a combined vaccine composed of: diphtheria formol toxoid; tetanus formol toxoid; individually purified antigenic components of Bordetella pertussis; suitable strains of human polioviruses 1, 2 and 3 grown in suitable cell cultures and inactivated by a suitable method; polysorbysribitol 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 diphtheriae and Clostridium tetani respectively. The vaccine contains either pertussis toxoid or a pertussis-toxin-like protein free from toxic properties produced by expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the toxin harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The acellular pertussis component may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of B. pertussis such as fimbrial-2 and fimbrial-3 antigens. The latter 2 antigens may be co-purified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

PRP is a linear copolymer composed of repeated units of 3-β-D-ribofuranosyl-1→1-ribitol-5-phosphate [C$_3$H$_9$O$_9$P], 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.

General Notices (1) apply to all monographs and other texts
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.

The content of bacterial endotoxins (2.6.14) in bulk purified diphtheria toxoid, tetanus toxoid, pertussis components, purified, inactivated monovalent poliovirus harvests and bulk PRP conjugate is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is less than the limit approved for the particular vaccine and, in any case, the contents are such that the final vaccine contains less than 100 IU per single human dose.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (2.6.9), and with the following test for specific toxicity of the diphtheria and tetanus components: 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, toxæmia or tetanus, the vaccine does not comply with the test. If more than 1 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.

During development studies and wherever revalidation is necessary, it shall be demonstrated by tests in animals that the vaccine induces a T-cell dependent B-cell immune response to PRP.

As part of consistency studies the assays of the diphtheria, tetanus, pertussis and poliomyelitis components are carried out on a suitable number of batches of vaccine reconstituted as for use. For subsequent routine control, the assays of these components may be carried out without mixing with the haemophilus component.

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.

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 (acellular, component, adsorbed) (1356), Poliomyelitis vaccine (inactivated) (0214) and Haemophilus type b conjugate vaccine (1219).

FINAL BULKS
The final bulk of the diphtheria, tetanus, pertussis and poliomyelitis components is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, bulk purified tetanus toxoid and bulk purified acellular pertussis components onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate and admixture of suitable quantities of purified, monovalent harvests of human polioviruses 1, 2 and 3 or a suitable quantity of a trivalent pool of such monovalent harvests. Suitable antimicrobial preservatives may be added. The final bulk of the haemophilus component is prepared by dilution of the bulk conjugate to the final concentration with a suitable diluent. A stabiliser may be added. Only final bulks that comply 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
The final bulk of the haemophilus component is freeze-dried. Only a final lot that is satisfactory with respect to the test for osmolality shown below and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided that the test for absence of residual pertussis toxin and irreversibility of pertussis toxoid, the test for antimicrobial preservative and the assay 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 and the purified monovalent harvests or the trivalent pool of polioviruses or 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, reconstituted where applicable, is within the limits approved for the particular preparation.

Free PRP. Unbound PRP is determined on the haemophilus component after removal of the conjugate, for example by anion-exchange, size-exclusion or hydrophobic chromatography, ultrafiltration or other validated methods. The amount of free PRP is not greater than that approved for the particular product.

IDENTIFICATION
Identification tests A, B, C and D are carried out using the vial containing the diphtheria, tetanus, pertussis and poliomyelitis components; identification test E is carried out on the vial containing the haemophilus component.

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 antitoxic, 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 pertussis components are identified by suitable immunochemical methods (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 specific antisera to the pertussis components of the vaccine.

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

E. The haemophilus component is identified by a suitable immunochemical method (2.7.1) for PRP.

TESTS
The tests for absence of residual pertussis toxin, irreversibility of pertussis toxoid, aluminium, free formaldehyde, antimicrobial preservative and sterility are carried out on the container with the diphtheria, tetanus, pertussis and poliomyelitis components; the tests for PRP content, water, sterility and pyrogens are carried out on the container with the haemophilus component.

Some tests for the haemophilus component may be carried out on the freeze-dried product rather than on the bulk conjugate where the freeze-drying process may affect the component to be tested.

Absence of residual pertussis toxin and irreversibility of pertussis toxoid. This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not fewer than 5 histamine-sensitive mice. Inject intraperitoneally into the first group twice the single human dose of the vaccine stored at 2–8 °C. Inject intraperitoneally into the second group twice the single human dose of the vaccine incubated at 37 °C for 4 weeks. Inject diluent into the third group of mice. After 5 days, inject into each mouse 2 mg of histamine base intraperitoneally in a volume not exceeding 0.5 ml and observe for 24 h. The test is invalid if 1 or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following histamine challenge. If 1 mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously threefold dilutions of a reference pertussis toxin preparation in phosphate-buffered saline solution containing 2 g/l of gelatin and challenge with histamine as above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

PRP: minimum 80 per cent of the amount of PRP stated on the label. PRP is determined either by assay of ribose (2.5.31) or phosphorus (2.5.18), by an immunochemical method (2.7.1) or by anion-exchange liquid chromatography (2.2.29) with pulsed-amperometric detection.

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 formaldehyde (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.

Water (2.3.12): maximum 3.0 per cent for the haemophilus component.

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

Pyrogens (2.6.8). It complies with the test for pyrogens. Inject per kilogram of the rabbit’s mass a quantity of the vaccine equivalent to: 1 µg of PRP for a vaccine with diphtheria toxoid or CRM 197 diphtheria protein as carrier; 0.1 µg of PRP for a vaccine with tetanus toxoid as carrier; 0.025 µg of PRP for a vaccine withOMP as a carrier.

ASSAY

Diphtheria component. Carry out one of the prescribed methods for the assay of diphtheria vaccine (adsorbed) (2.7.6). Unless otherwise justified and authorised, 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). The lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose.

Pertussis component. It complies with the assay of pertussis vaccine (acellular) (2.7.16).

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 Ph. Eur. 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 Ph. Eur. Units and intended for use in the assay of D-antigen. The Ph. Eur. Unit and the IU 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 names and amounts of the pertussis components 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,
- the number of micrograms of PRP per single human dose,
- the type and nominal amount of carrier protein per single human dose,
- 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,
- where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification.