Diphtheria vaccine (adsorbed) for adults and adolescents

DEFINITION
Diphtheria vaccine (adsorbed) for adults and adolescents is a preparation of diphtheria formol toxoid with a mineral adsorbent. The formol toxoid is prepared from the toxin produced by the growth of *Corynebacterium diphtheriae*. It shall have been demonstrated to the competent authority that the quantity of diphtheria toxoid used does not produce adverse reactions in subjects from the age groups for which the vaccine is intended.

PRODUCTION

GENERAL PROVISIONS

Specific toxicity. The production method is validated to demonstrate that the product, if tested, would comply with the following test: 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 toxemia, 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.

TESTS

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.

Sterility (2.6.1). The vaccine complies with the test for sterility.

ASSAY

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.

LABELLING

The label states:
- the minimum number of International Units 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.

01/2005:0646

DIPHTHERIA VACCINE (ADSORBED) FOR ADULTS AND ADOLESCENTS

Vaccinum diphtheriae adulti et adulescentis adsorbatum

BULK PURIFIED TOXOID
The bulk purified toxoid is prepared as described in the monograph on *Diphtheria vaccine (adsorbed)* (0443) and complies with the requirements prescribed therein.

FINAL BULK VACCINE
The final bulk vaccine is prepared by adsorption of a suitable quantity of bulk purified toxoid onto a mineral carrier such as hydrated aluminium phosphate or aluminium hydroxide; the resulting mixture is approximately isotonic with blood. Suitable antimicrobial preservatives may be added. Certain antimicrobial preservatives, particularly those of the phenolic type, adversely affect the antigenic activity and must not be used.

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

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 amount.

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

FINAL LOT
The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided 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 the free formaldehyde content has been determined on the bulk purified toxoid 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.

IDENTIFICATION
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. If a satisfactory result is not obtained with a vaccine adsorbed on aluminium hydroxide, carry out the test as follows. Centrifuge 15 ml of the vaccine to be examined and suspend the residue in 5 ml of a freshly prepared mixture of 1 volume of a 56 g/l solution of sodium edetate \( R \) and 49 volumes of a 90 g/l solution of disodium hydrogen phosphate \( R \). Maintain at 37 °C for not less than 6 h and centrifuge. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

TESTS

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.
Haemophilus type b conjugate vaccine

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

**ASSAY**

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 2 IU per single human dose.

**LABELLING**

The label states:
- the minimum number of International Units per single human dose,
- the name and the amount of the adsorbent,
- that the vaccine must be shaken before use,
- that the vaccine is not to be frozen.

**HAEMOPHILUS TYPE b CONJUGATE VACCINE**

Vaccinum haemophili stirpe b conjugatum

**DEFINITION**

Haemophilus type b conjugate vaccine is a liquid or freeze-dried preparation of a polysaccharide, derived from a suitable strain of *Haemophilus influenzae* type b, covalently bound to a carrier protein. The polysaccharide, polyribosylribitol phosphate, referred to as PRP, is a linear copolymer composed of repeated units of 3-β-D-ribofuranosyl(1→4)-ribitol-5-phosphate [(C10H19O12P)ₙ], with a defined molecular size. 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 haemophilus type b conjugate vaccines of adequate safety and immunogenicity in man. The production of PRP and of the carrier are based on seed-lot systems.

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

The stability of the final lot and relevant intermediates is evaluated using one or more indicator tests. Such tests may include determination of molecular size, determination of free PRP in the conjugate and the immunogenicity test in mice. Taking account of the results of the stability testing, release requirements are set for these indicator tests to ensure that the vaccine will be satisfactory at the end of the period of validity.

**BACTERIAL SEED LOTS**

The seed lots of *H. influenzae* type b are shown to be free from contamination by methods of suitable sensitivity. These may include inoculation into suitable media, examination of colony morphology, microscopic examination of Gram-stained smears and culture agglutination with suitable specific antisera.

No complex products of animal origin are included in the menstruum used for preservation of strain viability, either for freeze-drying or for frozen storage.

It is recommended that PRP produced by the seed lot be characterised using nuclear magnetic resonance spectrometry (2.2.33).

**H. INFLUENZAE TYPE b POLYSACCHARIDE (PRP)**

*H. influenzae* type b is grown in a liquid medium that does not contain high-molecular-mass polysaccharides; if any ingredient of the medium contains blood-group substances, the process shall be validated to demonstrate that after the purification step they are no longer detectable.

The bacterial purity of the culture is verified by methods of suitable sensitivity. These may include inoculation into suitable media, examination of colony morphology, microscopic examination of Gram-stained smears and culture agglutination with suitable specific antisera. The culture may be inactivated. PRP is separated from the culture medium and purified by a suitable method. Volatile matter, including water, in the purified polysaccharide is determined by a suitable method such as thermogravimetry (2.2.34); the result is used to calculate the results of certain tests with reference to the dried substance, as prescribed below.

Only PRP that complies with the following requirements may be used in the preparation of the conjugate.

**Identification.** PRP is identified by an immunochemical method (2.7.1) or other suitable method, for example 1H nuclear magnetic resonance spectrometry (2.2.33).

**Molecular-size distribution.** The percentage of PRP eluted before a given $K_v$ value or within a range of $K_v$ values is determined by size-exclusion chromatography (2.2.30); an acceptable value is established for the particular product and each batch of PRP must be shown to comply with this limit. Limits for currently approved products, using the indicated stationary phases, are shown for information in Table 1219-1. Where applicable, the molecular-size distribution is also determined after chemical modification of the polysaccharide.

Liquid chromatography (2.2.29) with multiple-angle laser light-scattering detection may also be used for determination of molecular-size distribution.

A validated determination of the degree of polymerisation or of the weight-average molecular weight and the dispersion of molecular masses may be used instead of the determination of molecular size distribution.

**Ribose (2.5.31).** Not less than 32 per cent, calculated with reference to the dried substance.

**Phosphorus (2.5.18):** 6.8 per cent to 9.0 per cent, calculated with reference to the dried substance.

**Protein (2.5.16).** Not more than 1.0 per cent, calculated with reference to the dried substance. Use sufficient PRP to allow detection of proteins at concentrations of 1 per cent or greater.

**Nucleic acid (2.5.17).** Not more than 1.0 per cent, calculated with reference to the dried substance.

**Bacterial endotoxins (2.6.14):** less than 25 IU per microgram of PRP.

**Residual reagents.** Where applicable, tests are carried out to determine residues of reagents used during inactivation and purification. An acceptable value for each reagent is established for the particular product and each batch of PRP must be shown to comply with this limit. Where validation studies have demonstrated removal of a residual reagent, the test on PRP may be omitted.