Pertussis vaccine (acellular, component, adsorbed)

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PERTUSSIS VACCINE (ACELLULAR, COMPONENT, ADSORBED)

Vaccinum pertussis sine cellulis ex elementis praeparatum adsorbatum

DEFINITION

Pertussis vaccine (acellular, component, adsorbed) is a preparation of individually prepared and purified antigenic components of Bordetella pertussis adsorbed on a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate.

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 latter harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The vaccine 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 copurified. 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.

PRODUCTION

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

Where a genetically modified form of B. pertussis is used for production consistency and genetic stability shall be established in conformity with the requirements of the monograph Products of recombinant DNA technology (0784).

Reference vaccine. A batch of 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 is preferably stabilised by a method that has been shown to have no significant effect on the assay procedure when the stabilised and non-stabilised batches are compared.

CHARACTERISATION OF COMPONENTS

During development of the vaccine, the production process shall be validated to demonstrate that it yields consistently individual components that comply with the following requirements; after demonstration of consistency, the tests need not be applied routinely to each batch.

Adenylate cyclase. Not more than 500 ng in the equivalent of 1 dose of the final vaccine, determined by immunoblot analysis or another suitable method.

Tracheal cytotoxin. Not more than 2 pmol in the equivalent of 1 dose of the final vaccine, determined by a suitable method such as a biological assay or liquid chromatography (2.2.29).

Absence of residual dermatonecrotic toxin. Inject intradermally into each of 3 unweaned mice, in a volume of 0.1 ml, the amount of component or antigenic fraction equivalent to 1 dose of the final vaccine. Observe for 48 h. No dermonecrotic reaction is demonstrable.

Specific properties. The components of the vaccine are analysed by one or more of the methods shown below in order to determine their identity and specific properties (activity per unit amount of protein) in comparison with reference preparations.

Pertussis toxin. Chinese hamster ovary (CHO) cell-clustering effect and haemagglutination as in vitro methods; lymphocytosis-promoting activity, histamine-sensitising activity and insulin secretory activity as in vivo methods. The toxin shows ADP-ribosyl transferase activity using transducin as the acceptor.

Filamentous haemagglutinin. Haemagglutination and inhibition by specific antibody.

Pertactin, fimbrial-2 and fimbrial-3 antigens. Reactivity with specific antibody.

Pertussis toxoid. The toxoid induces in animals production of antibodies capable of inhibiting all the properties of pertussis toxin.

PURIFIED COMPONENTS

Production of each component is based on a seed-lot system. The seed cultures from which toxin is prepared are managed to conserve or where necessary restore toxigenicity by deliberate selection.

None of the media used at any stage contains blood or blood products of human origin. Media used for the preparation of seed lots and inocula may contain blood or blood products of animal origin.

Pertussis toxoid and, where applicable, filamentous haemagglutinin and pertactin are purified and, after appropriate characterisation, detoxified using suitable chemical reagents, by a method that avoids reversion of the toxoid to toxin, particularly on storage or exposure to heat. Other components such as fimbrial-2 and fimbrial-3 antigens are purified either separately or together, characterised and shown to be free from toxic substances. The purification procedure is validated to demonstrate appropriate clearance of substances used during culture or purification.

The content of bacterial endotoxins (2.6.14) is determined to monitor the purification procedure and to limit the amount in the final vaccine. The limits applied for the individual components are such that the final vaccine contains less than 100 IU per single human dose.

Before detoxification, the purity of the components is determined by a suitable method such as polyacrylamide gel electrophoresis (PAGE) or liquid chromatography. SDS-PAGE or immunoblot analysis with specific monoclonal or polyclonal antibodies may be used to characterise subunits. Requirements are established for each individual product.

Only purified components that comply with the following requirements may be used in the preparation of the final bulk vaccine.

Sterility (2.6.1). Carry out the test for sterility using for each medium a quantity of purified component equivalent to not less than 100 doses.

Absence of residual pertussis toxin. This test is not necessary for the product obtained by genetic modification. Use a group of not fewer than 5 histamine-sensitive mice each weighing 18-26 g. Inject into each mouse the equivalent of 1 human dose intravenously or twice the human dose intraperitoneally, diluted to not more than 0.5 ml with phosphate-buffered saline solution containing 2 g/l of gelatin. Inject diluent into a second group of control mice.
Antimicrobial preservative requirements may be used in the preparation of the final lot. Only a final bulk vaccine that complies with the following suitable antimicrobial preservative may be added.

Aluminium hydroxide or hydrated aluminium phosphate. A nitrogen is within the limits established for the product.

Suitable method. The ratio of antigen content to protein content of the intended content.

Absence of residual pertussis toxin and irreversibility of pertussis toxoid by suitable chemical or physico-chemical method. The amount is not less than 85 per cent and not greater than 115 per cent of the amount of antimicrobial preservative by a suitable method.

Tests and Assay may be released for use. Provided that validation, for routine testing a single-dilution method may be used.

Residual detoxifying agents and other reagents. The content of residual detoxifying agents and other reagents is determined and shown to be below approved limits unless validation of the process has demonstrated acceptable clearance.

Antigen content. Determine the antigen content by a suitable immunochemical method (2.7.1) and protein nitrogen by sulphuric acid digestion (2.5.9) or another suitable method. The ratio of antigen content to protein nitrogen is within the limits established for the product.

FINAL BULK VACCINE

The vaccine is prepared by adsorption of suitable quantities of purified components, separately or together, onto aluminium hydroxide or hydrated aluminium phosphate. A suitable antimicrobial preservative may be added.

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 or physico-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.

FINtAL LOT

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 that the tests for absence of residual pertussis toxin and irreversibility of pertussis toxoid, antimicrobial preservative, free formaldehyde and the assay have been carried out with satisfactory results on the final bulk vaccine, these tests may be omitted on the final lot.

IDENTIFICATION

Subject the vaccine to a suitable desorption procedure such as the following: dissolve in the vaccine to be examined sufficient sodium citrate R to give a 10 g/l solution; maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. Examined by a suitable immunochemical method (2.7.1), the clear supernatant liquid reacts with specific antisera to the components stated on the label.

TESTS

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.

A validated test based on the clustering effect of the toxin for Chinese hamster ovary (CHO) cells may be used instead of the test in mice.

Residual detoxifying agents and other reagents. The content of residual detoxifying agents and other reagents is determined and shown to be below approved limits unless validation of the process has demonstrated acceptable clearance.

Antigen content. Determine the antigen content by a suitable immunochemical method (2.7.1) and protein nitrogen by sulphuric acid digestion (2.5.9) or another suitable method. The ratio of antigen content to protein nitrogen is within the limits established for the product.

ASSAY

The capacity of the vaccine to induce the formation of specific antibodies is compared with the same capacity of a reference preparation examined in parallel; antibodies are determined using suitable immunochemical methods (2.7.1) such as enzyme-linked immunosorbent assay (ELISA). The test in mice shown below uses a three-point model but, after validation, for routine testing a single-dilution method may be used.

Requirement. The capacity to induce antibodies is not significantly (P = 0.95) less than that of the reference vaccine. The following test model is given as an example of a method that has been found to be satisfactory.

Selection and distribution of test animals. Use in the test healthy mice (for example, CD1 strain) of the same stock 4 to 8 weeks old. Distribute the animals in 6 groups of a number appropriate to the requirements of the assay. Use 3 dilutions of the vaccine to be examined and 3 dilutions of a reference preparation and attribute each dilution to a group of mice. Inject intraperitoneally or subcutaneously into each mouse 0.5 ml of the dilution attributed to its group.

Collection of serum samples. 4 to 5 weeks after vaccination, bleed the mice individually under anaesthesia. Store the sera at −20 °C until tested for antibody content.

Antibody determination. Assay the individual sera for content of specific antibodies to each component using a validated method such as the ELISA test shown below.

ELISA test. Microtitre plates (poly(vinyl chloride) or polystyrene as appropriate for the specific antigen) are coated with the purified antigen at a concentration of 100 ng per well. After washing, unreacted sites are blocked by incubating with a solution of bovine serum albumin and then
washed. Two-fold dilutions of sera from mice immunised with test or reference vaccines are made on the plates. After incubation at 22-25 °C for 1 h, the plates are washed. A suitable solution of anti-mouse IgG enzyme conjugate is added to each well and incubated at 22-25 °C for 1 h. After washing, a substrate is added from which the bound enzyme conjugate liberates a chromophore which can be quantified by measurement of absorbance (2.2.25). The test conditions are designed to obtain a linear response for absorbance with respect to antibody content over the range of measurement used and absorbance values within the range 0.1 to 2.0.

A reference antisera of assigned potency is used in the test and serves as the basis for calculation of the antibody levels in test sera. A standardised control serum is also included in the test.

The test is not valid if:
- the value found for the control serum differs by more than 2 standard deviations from the assigned value,
- the confidence limits \(P = 0.95\) are less than 50 per cent or more than 200 per cent of the estimated potency.

Calculation. The antibody titres in the sera of mice immunised with reference and test vaccines are calculated and from the values obtained the potency of the test vaccine in relation to the reference vaccine is calculated by the usual statistical methods.

LABELLING

The label states:
- the names and amounts of the components present in the vaccine,
- where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification,
- the name and amount of the adsorbent,
- that the vaccine must be shaken before use,
- that the vaccine is not to be frozen.

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. A batch of 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 is preferably stabilised, by a method that has been shown to have no significant effect on the assay procedure when the stabilised and non-stabilised batches are compared.

CHARACTERISATION OF COMPONENTS

During development of the vaccine, the production process shall be validated to demonstrate that it yields consistently an antigenic fraction that complies with the following requirements: after demonstration of consistency, the tests need not be applied routinely to each batch.

Adenylyl cyclase. Not more than 500 ng in the equivalent of 1 dose of the final vaccine, determined by immunoblot analysis or another suitable method.

Tracheal cytotoxin. Not more than 2 pmol in the equivalent of 1 dose of the final vaccine, determined by a suitable method such as a biological assay or liquid chromatography (2.2.29).

Absence of residual dermonecrotic toxin. Inject intradermally into each of 3 unweaned mice, in a volume of 0.1 ml, the amount of antigenic fraction equivalent to 1 dose of the final vaccine. Observe for 48 h. No dermonecrotic reaction is demonstrable.

Specific properties. The antigenic fraction is analysed by one or more of the methods shown below in order to determine the identity and specific properties (activity per unit amount of protein) of its components in comparison with reference preparations.

Pertussis toxin. Chinese hamster ovary (CHO) cell-clustering effect and haemagglutination as in vitro methods; lymphocytosis-promoting activity, histamine-sensitising activity and insulin secretory activity as in vivo methods. The toxin shows ADP-ribosyl transferase activity using transducin as the acceptor.

Filamentous haemagglutinin. Haemagglutination and inhibition by specific antibody.

Pertactin, fimbrial-2 and fimbrial-3 antigens. Reactivity with specific antibody.

Pertussis toxoid. The toxoid induces in animals the production of antibodies capable of inhibiting all the properties of pertussis toxin.

PURIFIED ANTIGENIC FRACTION

Production of the antigenic fraction is based on a seed-lot system. The seed cultures are managed to conserve or, where necessary, restore toxigenicity by deliberate selection. None of the media used at any stage contains blood or blood products of human origin. Media used for the preparation of seed batches and inocula may contain blood or blood products of animal origin.

The antigenic fraction is purified and, after appropriate characterisation, detoxified using suitable reagents by a method that ensures minimal reversion of toxoid to toxin, particularly on storage or exposure to heat. The purification procedure is validated to demonstrate appropriate clearance of substances used during culture or purification.