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Preface

This module is part of the series *The Immunological Basis for Immunization*, which was initially developed in 1993 as a set of eight modules focusing on the vaccines included in the Expanded Programme on Immunization (EPI)\(^1\). In addition to a general immunology module, each of the seven other modules covered one of the vaccines recommended as part of the EPI programme — diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. The modules have become some of the most widely used documents in the field of immunization.

With the development of the Global Immunization Vision and Strategy (GIVS) (2005–2015) (http://www.who.int/vaccines-documents/DocsPDF05/GIVS_Final_EN.pdf) and the expansion of immunization programmes in general, as well as the large accumulation of new knowledge since 1993, the decision was taken to update and extend this series.

The main purpose of the modules — which are published as separate disease/vaccine-specific modules — is to give immunization managers and vaccination professionals a brief and easily-understood overview of the scientific basis of vaccination, and also of the immunological basis for the World Health Organization (WHO) recommendations on vaccine use that, since 1998, have been published in the *Vaccine Position Papers* (http://www.who.int/immunization/documents/positionpapers_intro/en/index.html).

WHO would like to thank all the people who were involved in the development of the initial *Immunological Basis for Immunization* series, as well as those involved in its updating, and the development of new modules.
This programme was established in 1974 with the main aim of providing immunization for children in developing countries.

1 This programme was established in 1974 with the main aim of providing immunization for children in developing countries.
Abbreviations & acronyms

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AC</td>
<td>adenylate cyclase</td>
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<tr>
<td>ACT</td>
<td>adenylate cyclase toxin</td>
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<td>AGG</td>
<td>agglutinogens</td>
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<tr>
<td>aP</td>
<td>acellular pertussis (vaccine)</td>
</tr>
<tr>
<td>BA</td>
<td>bacterial agglutination</td>
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<tr>
<td>BrkA</td>
<td>Bordetella resistance to killing genetic locus, frame A</td>
</tr>
<tr>
<td>BvgAS</td>
<td>complex virulence expression system</td>
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<td>CHO</td>
<td>Chinese hamster ovary (cells)</td>
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<tr>
<td>CMI</td>
<td>cell-mediated immunity</td>
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<tr>
<td>DNT</td>
<td>dermonecrotic toxin</td>
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<tr>
<td>DTaP</td>
<td>diphtheria–tetanus–acellular pertussis</td>
</tr>
<tr>
<td>DTP</td>
<td>diphtheria–tetanus–pertussis vaccine</td>
</tr>
<tr>
<td>DTwP</td>
<td>diphtheria–tetanus whole-cell pertussis (vaccine)</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>ELISPOT</td>
<td>enzyme-linked immunospot assay</td>
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<td>EPI</td>
<td>Expanded Programme on Immunization</td>
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<td>ESEN</td>
<td>European Sero-Epidemiology Network</td>
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<tr>
<td>FHA</td>
<td>filamentous haemagglutinin</td>
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<td>FIM</td>
<td>fimbriae</td>
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<td>GMT</td>
<td>geometric mean titre</td>
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<td>GSK</td>
<td>GlaxoSmithKline</td>
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<td>HCW</td>
<td>health-care worker</td>
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<td>Hlb</td>
<td>Haemophilus influenzae type B</td>
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<td>HLT</td>
<td>heat-labile toxin</td>
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<td>ICS</td>
<td>intracellular cytokine secretion</td>
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<td>Ig</td>
<td>immunoglobulin</td>
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<tr>
<td>IPV</td>
<td>inactivated polio vaccine</td>
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<td>Lf</td>
<td>flocculation units of toxoid</td>
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<td>LOS</td>
<td>lipooligosaccharide</td>
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<td>LPS</td>
<td>lipopolysaccharide</td>
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<td>MRC</td>
<td>Medical Research Council</td>
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<td>NACI</td>
<td>National Advisory Committee on Immunization</td>
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<td>NIBSC</td>
<td>National Institute for Biological Standards &amp; Control</td>
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<td>NIH</td>
<td>National Institutes of Health</td>
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<tr>
<td>NT</td>
<td>neutralization test</td>
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<td>OMP</td>
<td>outer membrane protein</td>
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<td>PRN</td>
<td>pertactin</td>
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<td>PRP</td>
<td>polyribosyl-ribitol-phosphate</td>
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<tr>
<td>PT</td>
<td>pertussis toxin</td>
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<tr>
<td>RGD</td>
<td>arginine-glycine-aspartic acid</td>
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<tr>
<td>SphB1</td>
<td>serine-protease/lipoprotein</td>
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<tr>
<td>TcfA</td>
<td>tracheal colonization factor</td>
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<tr>
<td>TCT</td>
<td>tracheal cytotoxin</td>
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<tr>
<td>Tdap</td>
<td>DTaP with reduced antigen content</td>
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<td>VE</td>
<td>vaccine efficacy</td>
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<td>WHO</td>
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<td>wP</td>
<td>whole-cell pertussis (vaccine)</td>
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**Pertussis**

*Bordetella pertussis* is a strictly human pathogen with multiple biological activities. The bacteria are transmitted by droplets and the infectious dose is small in immunologically naïve patients. Pertussis infection begins with the attachment of *B. pertussis* to the ciliated epithelium of the respiratory tract; the subsequent manifestations are thought to be the result of the interplay between various virulence factors (toxins) of the organism (Table 1, Figure 1). Irrespective of high vaccination coverage, *B. pertussis* circulates in all countries; reinfections are common and occur throughout a person’s lifetime.

The genomes of *B. pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. avium* have been sequenced and are available publicly (Parkhill et al., 2003; Sebaihia et al., 2006). *B. pertussis* and *B. parapertussis* appear to have emerged rather recently from a common *B. bronchiseptica*-like ancestor. When compared to *B. bronchiseptica*, large parts of the genome of *B. pertussis* and *B. parapertussis* were inactivated or lost during adaptation to the human host. Compared to other human pathogens, isolates of *B. pertussis* show only small genomic heterogeneity, suggesting a more recent development as a human pathogen (Brinig et al., 2006; King et al., 2006).

**Antigens of Bordetella pertussis**

Recent research has contributed much to the understanding of the structure and function of *Bordetella pertussis*, and has lead to the development of acellular pertussis (aP) vaccines. For an in-depth survey into the biology of *B. pertussis* see Matoo & Cherry, 2005 and Locht, 2007.

The virulence factors are controlled by a complex virulence expression system (BvgAS). BvgA is a DNA-binding response regulator, and BvgS is a 135-kDa transmembrane sensor kinase. The virulence factors under the regulation of the BvgAS system may be functionally characterized as adhesins and autotransporters (filamentous haemagglutinin, fimbriae, pertactin, and tracheal colonization factor), toxins (pertussis toxin, adenylate cyclase toxin, dermonecrotic toxin, tracheal cytotoxin, and lipopolysaccharide), and other antigens. Decades ago, it was observed that *B. pertussis* can display different “phases” in response to the environment; these were called phases I, II, and III. Thus, *B. pertussis*, similar to other Bordetellae, is capable of responding to the environment by switching from the X-mode (all virulence factors expressed) to the I mode, in which some virulence factors are suppressed, and theoretically also into the C mode, where almost no virulence factors are expressed and which was demonstrated as a starvation survival mode in *B. bronchiseptica*. For a summary see Locht, 2007.

**Pertussis toxin**

The best known toxin of *B. pertussis* is pertussis toxin (PT), which has several biological activities. PT, similar to other bacterial toxins, is a typical AB toxin consisting of two main subunits; an enzymatically active A (S1) subunit and a B (S2-S5) oligomer which binds to receptors on target cells. The B oligomer has no enzymatic activity, but is required for efficient binding of the toxin to cells, and allows the S1 enzymatic subunit to reach the site of action within the target cell. S1 is an ADP-riboseyltransferase and ribosylates G proteins (Pittman, 1979; Burns, 1988; Kerr & Matthews, 2000). Antibodies to PT develop after natural infection or vaccination. PT can be chemically or genetically inactivated, but still retain its immunogenicity. Other biological activities of PT include histamine sensitization, induction of lymphocytosis, insulin secretion and modification of immune responses. PT is produced only by *B. pertussis* although the genome of other *Bordetella* Spp. such as *B.*
parapertussis and B. bronchiseptica contain a nonfunctional ptx gene. Thus, PT is the only antigen specific for B. pertussis.

Sequencing the ptx genes in circulating and historic strains has shown that PT displays some degree of polymorphism with different ptx genes named, ptx1, ptx2 etc. Most circulating strains are ptx1. Some polymorphism is also observed in the promoter of the PT-operon, which could modify the expression of the toxin (Mooi et al., 2009)

PT is believed critical to the action of B. pertussis and is a component of all aP vaccines.

**Adenylate cyclase toxin**

*B. pertussis* adenylate cyclase toxin (ACT), a hemolysin with enzymatic activity, is secreted in high concentration into the extracytoplasmatic space. It belongs to the family of bacterial calmodulin activated repeats-in-toxin (RTX) toxins. By close contact between the bacteria and the cells, ACT enters the cells and inhibits the microbicidal and cytotoxic function of neutrophils, monocytes, and natural killer cells. ACT probably contributes to clinical pertussis through impairment of host defenses or through a direct effect on the respiratory mucosa (Hewlett et al., 2006). ACT is produced during pertussis infection in humans, and it induces production of anti-AC antibodies that may persist into adulthood (Arcienaga et al., 1991; Cherry et al., 2004). Antibodies may also be produced in low titres after vaccination with whole-cell vaccines (Farfel et al., 1990). Adenylate cyclase toxin is also produced by *B. parapertussis* and *B. bronchiseptica*. Inactivated ACT toxin is not a component of aP vaccines.

**Lipopolysaccharide**

*B. pertussis* organisms produce a lipopolysaccharide endotoxin (LPS), similar to other gram-negative bacteria. By contrast with other Bordetella spp., the *B. pertussis* LPS lacks a long O-antigenic chain, and is also called lipooligosaccharide (LOS). LOS is probably responsible for some of the adverse reactions in children following whole cell (wP) pertussis immunization, and has antigenic (although not protective) and adjuvant properties. The amount of LOS in wP vaccines has been shown to be largely associated with the frequency of fever after vaccination (Baraff et al., 1989). LOS was also recognized as one of the agglutinogens, formerly called AGG1. *B. pertussis* LOS is generally not contained in aP vaccines, depending on the purification processes of the vaccines.

**Dermonecrotic toxin, heat-labile toxin**

Dermonecrotic toxin (DNT), one of the first discovered virulence factors of *B. pertussis*, induces dermal necrosis in mice when injected intradermally. This heat-labile toxin (HLT) is a 160 kDa secreted protein, the structure of which is compatible with an A-B model of bacterial toxins. It induces necrosis of various cell types in vitro. However, in a mouse model, variants of *B. pertussis* lacking dermonecrotic toxin are no less virulent than wild strains. DNT is not contained in aP vaccines.

**Tracheal cytotoxin**

Tracheal cytotoxin (TCT) is a fragment of bacterial peptidoglycan that causes loss of ciliated cells and reduction of ciliary activity in vitro, possibly related to an increase in nitric oxide and/or IL-1α. In its structure TCT resembles the biological response modifier, muramyl dipeptide (Flak & Goldman, 1999). TCT is not contained in aP vaccines.
Filamentous haemagglutinin

Filamentous haemagglutinin (FHA) is a large hairpin-shaped (molecular-weight 220 kDa) surface-associated and secreted protein. FHA has no enzymatic activity but plays a major role in the initial colonization of *B. pertussis* by mediating the adhesion of *B. pertussis* to the ciliated epithelium of the upper respiratory tract. FHA belongs to the “two-partner secretion” systems of bacterial excreted proteins, in which a transporter protein, subtilisin-like serine-protease/lipoprotein (SphB1), is responsible for the recognition and transport of FHA. FHA is produced by *B. pertussis*, *B. parapertussis* and *B. bronchiseptica*, and it cross-reacts with structures from other bacteria (Mattoo & Cherry, 2005). Genomic studies of the *fha*-genes have shown almost no heterogeneity among different strains (Mooi & Greef, 2007).

FHA is contained in most aP vaccines.

Pertactin

Pertactin (PRN), an autotransporter, is a 68-70 kDa surface protein that mediates eukaryotic cell binding by its Arg-Gly-Asp (RGD) motif, and is also produced by other pathogenic members of the genus *Bordetella*. PRN is highly immunogenic (Shahin et al., 1990).

The *prn* genes are among the most polymorphic in the *B. pertussis* genome, and various *prn* types (prn1-prn11) have been identified (Mooi & Greef, 2007). Changes in *prn* types have been suspected by some workers in the Netherlands to contribute to a reduced vaccine efficacy in whole-cell vaccines (Mooi et al., 2001). The *prn* type of *B. pertussis* strain Tohama, from which all current aP vaccines are derived, expresses PRN1. However, most strains now circulating are other *prn* types, predominantly PRN2 and PRN3. This variability does not appear to have interfered to a measurable degree with the effectiveness of aP vaccines. By contrast to antibodies produced in rabbits, PRN antibodies to those epitopes that are influenced by polymorphisms have not been detected in humans (Hijnen et al., 2008).

PRN is an antigen in many aP vaccines. Low amounts of PRN, sufficient to induce immune responses, are present in some two-component (PT and FHA) aP vaccines thought not to contain PRN (Edwards & Decker, 2008).

TcfA and other autotransporters

The tracheal colonization factor (TcfA) is an autotransporter implicated in the pathogenesis in a murine model. The role of other autotransporters such Vag8, a 95kDa membrane protein, warrant further studies on their role in human pertussis. Antibodies to TcfA develop slowly after primary infection, but rapid increases are noted on secondary exposure. These autotransporters are not contained in aP vaccines.

Fimbriae

Fimbriae (FIM) types 2 and 3 also represent serotype-specific agglutinogens (AGG) and are important surface components involved in colonizing the respiratory mucosa. FIM 2 and 3 contained in whole-cell pertussis (wp) vaccines are believed to contribute to protective efficacy, and the WHO requirements for pertussis vaccine licensure require the demonstration of the presence of such agglutinogens (WHO, 1990). Most manufacturers use several strains of *B. pertussis* for the production of wp vaccines to ensure the presence of both types of fimbriae (Kudelski et al., 1978), although some manufacturers base their production on only one strain (Huovila et al., 1982).
Isolates of *B. pertussis* can display FIM2, FIM3 or both on their surface. It was observed very early on that the FIM type of circulating strains could change over time (Mooi et al., 2007). However, whether or not the changes in FIM2 and FIM3 types are induced by vaccination pressure is still under discussion. Although the *fim* genes are rather preserved, polymorphisms among FIM antigens have been found, and one structure, FimD, is common to all fimbriae.

FIM 2/3 are antigens in some aP vaccines. FIM antigens may be present in minute amounts in antigen preparations of aP vaccines thought not to contain FIM.

**Bordetella resistance to killing genetic locus, frame A**

*Bordetella* resistance to killing genetic locus, frame A (BrkA), a 73 kDa protein with a large 30 kDa outer surface domain, protects the bacteria against being killed by the classical pathway of complement. Antibodies to BrkA develop slowly after primary infection, but rapid increases are noted on secondary exposure. This has been suggested as a possible explanation for the milder clinical course in repeat pertussis infections (Weiss et al., 1999). BrkA is not contained in aP vaccines.

**Other structural components**

Envelope-associated capsule proteins of *B. pertussis* occupy an exposed location on the bacterium and may therefore be accessible to the host immune system. The bacteria also express flagella and type IV pili on their surface.

Our understanding of the role of particular components of *B. pertussis* in the pathogenesis and immunity to the disease is impaired by the lack of an animal model which is equivalent to clinical pertussis in humans.

**Pertussis vaccines**

**Whole-cell pertussis vaccines**

Whole-cell pertussis (wP) vaccines contain various amounts of whole non-viable bacterial cells. All antigens and virulence factors described above, such as PT, ACT, LOS, FHA, and AGG can be components of wP vaccines.

wP vaccines are produced in many countries, and the WHO has set quality requirements for production and lot release (WHO, 1990). wP vaccines are produced by growing bacteria in standardized liquid synthetic media. The bacteria are then killed, adjusted to a certain density (i.e. number of cells), mostly adsorbed to aluminium salts, and a preservative is added. The production process and the composition of strains may vary from producer to producer. The potency of wP vaccines is mostly controlled by an intracerebral mouse challenge test developed in the 1940s (Kendrick et al., 1947). Although this test has been used for a long time, it is not clear what type of the murine immune response it measures.

Considerable variation has been found in the amount of FHA and PT in different wP vaccines. Measured as antigen, FHA ranges between 0 and 1.6 μg per dose, and total biologically-active PT has been reported to be in the range of 0.02 to 0.68 μg per dose (Ashworth et al., 1983). The amount of AGG2 in Wellcome wP vaccine was estimated to be 4.7 μg per single dose (Ashworth et al., 1983).

The amount of LOS in wP vaccines ranges from 0.9 to 2.8 μg per ml, and most has been found to exist as free, not cell-bound toxin. The release of LOS from cells during storage of
vaccine is quite rapid; in the first few weeks 35% to 50% of the LOS is released, and after five to six months 60% to 80% of LOS is released (Ibsen et al., 1988).

Although the production process of wP vaccines seems to be simple and standardized, significant differences have been observed in the immunogenicity and efficacy of wP vaccines from different producers (Bellalou & Relyveld, 1984). wP vaccines were included as a comparator in the trials on aP vaccines during the 1990s. The studies used different designs, and so the estimates of efficacy for the wP groups cannot directly be compared. A German whole-cell vaccine showed vaccine efficacy estimates (VE) of 98% and 96% in two studies. One American vaccine had an estimate of 83% in another study done in Germany. A French-made vaccine had a VE estimate of 96% in a study in Senegal. By contrast, an American whole-cell vaccine that had passed the potency tests was found to be only 36% efficacious in Italy and only 48% in Sweden.

Similar low effectiveness estimates were reported from other countries by using surveillance data. In the Netherlands, data from 1997 of the local vaccine against bacteriological proven pertussis suggested an effectiveness of 51% (de Melker et al., 2000a). In a case-control study in Canada in children aged four years and over, vaccine effectiveness against laboratory-confirmed pertussis was only 57% (Bentel-Enchil et al., 1997).

wP vaccines are not licensed for routine use in adolescents and adults.

**Acellular pertussis vaccines**

Recognition of the roles of PT, FHA, and AGG in the pathogenesis of, and immunity against, pertussis, together with concerns about frequent local side-effects, as well as public anxiety about the safety of wP vaccines, prompted the development of aP vaccines. All aP vaccines are associated with significantly lesser side-effects, and thus the replacement of the wP vaccines was mainly driven by the safety-profile of these vaccines. The other important advantage of the aP vaccines is the reproducible production process with its use of purified antigens and the removal of LPS and other parts of the bacterial cell wall during the purification of soluble antigenic material.

Initial aP vaccines were prepared through a co-purification process, and they contained a substantial predominance of FHA over PT (30 to 40 μg of FHA and about 5 μg PT per dose), and a small amount of AGG (about 1 μg per dose) (Aoyama et al., 1989). These aP vaccines were studied in Japan and in Europe (Aoyama et al., 1989; Mortimer et al., 1990; Kimura & Kuno-Saki 1990; Tomoda et al., 1991; Stehr et al., 1998. The second type of aP vaccines purified the antigens separately and contained equal amounts of FHA and PT (usually 12.5 to 24 μg per dose). They were initially licensed and used in Japan for children over two years of age, based on immunogenicity data without an efficacy study.

To evaluate multiple new aP vaccine candidates, a multicentre aP trial was conducted that assessed the safety and immunogenicity of 13 candidate aP vaccines, and two wP vaccines (Decker & Edwards, 1995). Taking into account the results of this immunogenicity study, in subsequent years an array of field studies was performed (Table 2) (Edwards & Decker, 2008). Although the efficacy trials differed significantly in vaccination times, design, case definition, and technical aspects such as culture and serology, it was attempted to put the results of all the studies into a synopsis (Edwards & Decker 2008). Unfortunately, all the trials failed to identify reliable serologic correlates for clinical protection of the individual (see below).
As a result of these studies, aP vaccines were licensed in most countries for primary immunization and for booster immunization. Most licensed aP vaccines contain between one and four/five separately purified antigens (PT, FHA, PRN, FIM 2/3). A long-term surveillance of the effectiveness of the aP vaccines was initiated in Sweden, starting after the completion of efficacy trials there. Although the vaccines used in different parts of Sweden differed, the overall reduction of cases in all vaccinated cohorts was maintained; the surveillance ended in 2008 (http://www.smittskyddsinstitutet.se/upload/Publikationer/SMI-rapport-2006-4.pdf).

Compared to wP vaccines, aP vaccines are associated with a significantly reduced frequency of systematic reactions (fever, vomiting, fretfulness, anorexia) and local reactions (swelling, redness, warmth, tenderness). The various efficacy trials in the 1990s and the subsequent post-marketing surveillance, as well as national surveillance systems, such as the Vaccine Adverse Event Reporting System (VAERS) in the United States of America, have produced a large amount of data about the reduced reactogenicity of aP vaccines. Various reviews have summarized the side-effects of aP vaccines in infants (Zhou et al., 2003). Of particular concern was the observation of gross limb swelling after vaccination, which was not painful, and did not interfere with overall health, but troubled parents. A systematic review (Rennels, 2003) showed that this type of side-effect was not typical for aP vaccines, but that all paediatric vaccines produced limb swelling in varying frequency. However, this reaction was seen more frequently with aP vaccines than with wP vaccines. A recent study gave a fifth dose of aP vaccine to recipients that had experienced limb swelling after the fourth dose, and only 20% experienced a recurrence of limb swelling (Rennels et al., 2008). Some cohorts of aP vaccine study participants have now received up to six doses of aP vaccine, and the frequency of limb swelling was not reported to increase after the sixth dose (Zepp et al., 2006).

Due to their safety profile, aP vaccines also offered the possibility of vaccinating older children, adolescents and adults. Recent developments focus on aP vaccines with reduced antigen content (i.e. 50% or less of the infant formulation) to further reduce unwanted side-effects. These vaccines have also undergone extensive studies relating to their immunogenicity and side-effects. One of the reduced-dose aP vaccines was tested for efficacy in a trial among American adolescents and adults and was found to have a point estimate of efficacy of 92% (95% CI: 32%–99%) (Le et al., 2004. In another study in British adolescents, both reduced-dose vaccines, in combination with tetanus and diphtheria toxoid (Tdap and Tdap-IPV, respectively), were both immunogenic and safe (Southern et al., 2005). The effectiveness of giving combined TdaP-vaccines was recently shown in Australia, and with a point estimate of 0.85, it was similar to the efficacy trial mentioned above (Rank et al., 2009). When Tdap was recommended for adolescents, it was observed that the frequency of post-vaccination syncopes was slightly higher in female vaccinees (CDC, 2008).

**Combination vaccines**

In this context, monovalent is used to indicate that the vaccine contains only pertussis antigens, and monocomponent is used to indicate that the vaccine contains only one single pertussis antigen.

wP vaccines have, for a long time, been combined with tetanus and diphtheria toxoid into the diphtheria–tetanus whole-cell pertussis (DTwP) vaccine. However, monovalent wP vaccines are still available in a few countries. Most aP vaccines are combined with other antigens into combination vaccines, and no monovalent aP vaccine is available in the Americas or the European Union. A monovalent aP vaccine is available in China.

Apart from problems in the production of combination vaccines, other concerns arise from possible interferences between antigens, as, for instance, it has been shown that the
geometric mean titres of antibodies against *H. influenzae* type b polysaccharide are significantly lower in vaccinees who received combination vaccines. Secondly, combination vaccines have generated regulatory concerns, because their safety and effectiveness may be more difficult to monitor and regulate than single component vaccines (Decker, Edwards & Bogaerts, 2008). Immunologic theory suggests that the simultaneous exposure of the immune system to multiple conjugate antigens (such as Hib, Streptococcus pneumoniae, and *Neisseria meningitis*), could result in either enhanced or suppressed immune responses. Suppression is assumed to occur when a specific carrier for a polysaccharide is given more than once; this phenomenon is called carrier-induced epitopic suppression.

Combination vaccines are licensed in the assumption that combining their antigens does not interfere with their safety, immunogenicity and effectiveness. This is mostly not monitored by efficacy studies, but by non-inferiority studies comparing the immunogenicity of separately-administered licensed vaccines. A recent example, however, may illustrate possible problems: DTaP-Hib-combination vaccines were introduced in the United Kingdom when another combination with wP was not available. The United Kingdom used a 2-, 3-, 4-month schedule for primary immunization without a booster in the second year of life. Surveillance showed that invasive Hib disease increased, especially in the recipients of the DTaP-Hib combination. However, when a booster dose was introduced, invasive Hib disease fell rapidly to low levels, as experienced in other countries, such as Germany, that used a booster in the second year of life (Health Protection Agency; 2008; Kalies et al., 2004). These, and other experiences, suggest that sufficient post marketing surveillance will be necessary if vaccination plans are changed from non-combined to combination vaccines.

Safety of combination vaccines has, so far, been reassuring, as none of the combination vaccines has produced side-effects that were not observed with any of its components. Overall, combination vaccines tend to have slightly more local side-effects when compared to the injection of their antigens separately. However, the reduction of the number of injections, especially in infants, is regarded as a significant advantage for these products. Furthermore, it was observed that the use of combination vaccines improved the timeliness of vaccination in both German and American infants (Kalies et al., 2006; Happe at al., 2009). Overall, combination vaccines, especially for primary immunization of infants, have been very successful, with a good safety profile, and are used in most parts of the world.

**Combination vaccine including whole-cell pertussis components**

The amount of pertussis antigens is low compared with the levels of protein in the tetanus and diphtheria toxoids in a dose of DTwP vaccine. A dose of DTwP vaccine mostly contains 20 Lf of diphtheria toxoid and 10 Lf or more of tetanus toxoid. These amounts of toxoid provide 80 µg of diphtheria antigen and 40 µg of tetanus antigen per vaccine dose.

wP vaccines, together with tetanus and diphtheria toxoids, were the “building blocks” for all other infant combination vaccines. DTwP vaccines have been combined with *H. influenzae* type b polysaccharide, with HBs-antigen, with inactivated polio vaccines (IPV) and, experimentally, with *Neisseria meningitis* type C vaccine. Many of these combination products are used for primary immunization of infants. (Decker, Edwards & Bogaerts, 2008). Most immune responses to the different antigens were similar, when antigens were either injected separately or as a combination. Antibodies to the polyribosyl-ribitol-phosphate (PRP) of Hib, however, were reproducibly lower when the antigen was given in a combination vaccine. However, these differences may be clinically irrelevant, or relevant, depending on the particular immunization scheme.
Combinations with acellular pertussis vaccine

aP vaccines were primarily combined with tetanus and diphtheria toxoids. Similar to wP vaccines, aP vaccines have also been combined with *H. influenzae* type b polysaccharide, with HBs-antigen, with IPV vaccines and, experimentally, with *N. meningitis* type C vaccine. Many of these combination products, with antigens from five or six different microorganisms, are used for primary immunization of infants. For a more detailed discussion on the immunogenicity of aP combination vaccines, see Decker, Edwards & Bogaerts, 2008.

Apart from combination vaccines for primary immunization, reduced dose combination vaccines have been developed for booster immunization. These vaccines contain about one third of the antigen content of those products for primary immunization; they are combined with reduced dose tetanus and diphtheria toxoids to form Tdap products. For certain purposes, the Tdap vaccines are combined with IPV or other antigens.

Although the efficacy study of an aP vaccine for adults and adolescents was done with a non-combined vaccine (Le et al., 2004), all immunogenicity data and a recent effectiveness study of reduced dose combination vaccines with pertussis components (Rank et al., 2009) suggest that they are as effective as a non-combined product.

Measuring the immune response to *B. pertussis* antigens

Although wP vaccines have been used successfully for decades, there are no reliable measures of immunity to pertussis. Furthermore, although many of the serological techniques have proved useful as diagnostic procedures, it is unclear whether any of them or a combination of which is a measure of protection to pertussis in the individual (Table 3).

Another crucial point is the standardization of detection methods to make results comparable. These standardizations would include methodology, antigens and reference materials, which so far have only been partly achieved for enzyme-linked immunosorbent assay (ELISA) methodology (Giammanaco et al., 2008; Tondella et al., 2009) A WHO reference preparation for human pertussis serology has been developed and is available from the National Institute for Biological Standards & Control (NIBSC) (Xing et al., 2009). All the methods used for measuring the immune response to *B. pertussis* antigens are also being used as tools to diagnose the disease, and commercially distributed tests of variable quality are available (Koesters et al., 2000). Several of these methods are described below.

Bacterial agglutination test

The bacterial agglutination (BA) test, the first method developed to measure pertussis antibodies, employs a simple technique for measuring mainly IgM-antibodies induced by the fimbriae, PRN and LPS of *B. pertussis*. Early studies by Miller et al. (1943) and Sako (1947) suggested some correlation of agglutinins with immunity; vaccinated children with high agglutinin titres were protected from household exposure to pertussis. Recent studies have neither confirmed nor refuted this observation. After infection, agglutinins are not regularly produced. After vaccination with whole-cell vaccines, agglutinins are often produced, but vaccinees without agglutinating antibody have been shown to be protective. For example, the first "acellular" vaccine (based on sonically disintegrated *B. pertussis* cells called Pillemer antigen) was shown to provide strong protection in children, but it had a weak capacity to stimulate production of agglutinins in mice and children (MRC, 1959).

The BA test suffers from low sensitivity and it has not been standardized. The agglutinin titres strongly depend on the bacterial strain used (Wilkins et al., 1971; Blumberg et al., 1992). BA antibodies correlate best with antibodies to FIM determined by the ELISA test.
There is a better correlation between the results of these tests when the BA titre is above 1:320 than at lower BA titres.

**Enzyme-linked immunosorbent assay**

The enzyme-linked immunosorbent assay (ELISA) uses purified protein antigens of *B. pertussis* (such as FHA, PT, PRN or FIM 2/3) to measure serum immunoglobulin IgG and IgA responses following disease or vaccination (Granstrom et al., 1982; Ashworth et al., 1983; Burstyn et al., 1983; Mertsola et al., 1983; Baraff et al., 1984; Granstrom et al., 1988; Stroffolini et al., 1989; Thomas et al., 1989b; Zackrison et al., 1990; Lynn et al., 1996). In addition, ELISAs have also been used to measure antibodies in saliva (Litt et al., 2006). The ELISA test is sensitive, specific, relatively cheap, and requires only a small amount of serum. The accuracy of the test depends on the purity of the antigens involved. With mixed preparations (whole bacteria, sonicate or extract of bacteria), it is not possible to identify the particular antigens to which the antibody response is directed (Thomas et al., 1989a).

The use of ELISA to quantify anti-pertussis toxin (PT) antibody levels can be performed with paired (acute and convalescent phase) or single serum samples (Cherry et al., 2005; Mattoo & Cherry, 2005). Paired sample serology is a standardized method of diagnosing pertussis, being the most sensitive and specific. However, the need to collect two samples and to wait several weeks for the result, makes it impractical for routine diagnosis. For this reason, single sample serology has been developed and IgG anti-PT serological cut-off values have been determined in a number of laboratories; this technique has been shown to provide good sensitivity and specificity to determine cases in adolescents and adults (Marchant et al., 1994; Wirsing von König et al., 1999 and 2002; de Melker et al., 2000; Baughman et al., 2004 Prince et al., 2006).

All studies that gave recommendations about the use of serology in pertussis diagnosis were performed in populations vaccinated with DTwP vaccines. Diphtheria–tetanus–acellular pertussis (DTaP) vaccines are now being used in many countries. DTaP vaccines induce different immune responses than DTwP, resulting in higher titres of antibody. (Greco et al., 1996; Olin et al., 1997). Antibody responses to specific antigens are often different in DTaP vaccines compared with DTwP vaccines and therefore last longer (Greco et al., 1996; Olin et al., 1997; Guiso et al., 2007; Riffelmann et al., 2009). For this reason, recommendations regarding serological cut-offs for single sample serology may require monitoring of their sensitivity and specificity when the vaccination schedule is changed.

There are several additional problems with pertussis serology, such as the lack of distinction between vaccine- and infection-induced immunological responses (symptomatic or asymptomatic infection), the absence of validated commercial assays, and commercial availability of purified pertussis toxin and other antigens.

**Immunoblot assays**

Immunoblot techniques for measuring antibodies to *B. pertussis* were developed in the late 1980s (Thomas et al., 1989a). Since then these assays have been used in pertussis diagnosis (Reed et al., 1988; Guiso et al., 1993), but have limitations. Immunoblots cannot readily quantify antibodies. In most assays, purified pertussis antigens are used, which may be used more easily in an ELISA format; no typical pattern of immunoblot reactivity has been evaluated if a whole-cell lysate is used for this technique.

**Other tests for measuring antibodies**

Flow cytometry-based serological tests using multicoloured beads have been applied to pertussis serology, offering the advantage of measuring various antibody specificities in a
single test. These tests were found to correlate well with standardized ELISA procedures (Pickering et al., 2002; Prince et al., 2006, Reder et al., 2008, van Gageldonk et al., 2008).

The in vitro neutralization test for antibodies to PT is conducted using microtitre cultures of Chinese hamster ovary (CHO) cells. PT induces a distinct cytopathogenic effect resulting in the clustering of CHO cells in the microplate culture. Only a small amount of PT (about 1 ng) is needed to produce the clustering of CHO cells. The addition of sera to the microcultures allows the in vitro neutralization of the toxin to be measured (Gillienius et al., 1985; Granstrom et al., 1985). The neutralization test (NT) is laborious, requires tissue-culture facilities, and involves subjective readings. Although the titres of NT tests correlate well with the results of IgG-anti-PT ELISA, the NT is significantly less sensitive for the diagnosis of pertussis than measuring the IgG response to PT by ELISA. Furthermore, not all patients develop measurable neutralizing antibodies after clinical and culture-confirmed whooping cough (Granstrom et al., 1988).

Other serological methods, such as indirect haemagglutination, bactericidal reaction, immunodiffusion, and complement fixation have also been used, but overall have not gained wide acceptance (for a review see Onorato & Wassilak, 1987).

**Tests for cell-mediated immunity**

As antibody testing does not reliably predict a protective immunity, many studies have focused on measuring cell-mediated immunity to antigens of *B. pertussis* using various methods (Ryan et al., 1997).

Lymphocyte proliferation assays have been mostly used in analyzing cell-mediated immunity to pertussis. Mononuclear cells are cultured with various pertussis antigens and with polyclonal stimulants as controls. The proliferation of the cells is measured by the ingestion of radiolabeled nucleotides into the cells. Results are given as fold increases when compared to the control without stimulants.

The cytotoxic T-cell response can be measured by chromium release assays, which have not been used for pertussis. Newer tests measure the cytokine secretion, either by directly measuring the cytokines in the culture supernatant intracellular cytokine secretion (ICS), or by counting the cytokine producing cells by enzyme-linked immunospot assay (ELISPOT). These assays have also been used for testing immunity to *B. pertussis* antigens (He et al., 1998; Tran Minh et al., 1999; Mills, 2001; Rieber et al., 2008).

Other assays measuring cell-mediated immunity (CMI), such as tetramer assays and polychromatic flow cytometry, have not yet been applied to study the response to *B. pertussis* antigens in humans.

The findings of the different assays are difficult to compare, firstly because various biological activities are measured, but also because the assays are not very well standardized and they can be influenced, among other parameters, by the age and stability of the cells, by the method by which the cells are purified, by the anticoagulant, by the type and source of antigen used and by other factors.

Cell-mediated immunity, as measured by most methods, seems to be long-lived, but has not been found to be a reliable correlate of protection against reinfection (Cherry et al., 2005).
Immune responses after exposure to *Bordetella pertussis*

The natural course of pertussis disease is influenced by the age-specific proportion of susceptible and resistant persons in the community (Galazka, 1992). It is also important to bear in mind that neither infection nor vaccination confers long-lasting immunity to subsequent infection or disease.

Although no specific antibody against antigens of *B. pertussis* has been convincingly shown to confer immunity against the disease, the prevalence of these antibodies at different ages can be used as an index of the exposure to pertussis antigens. A number of seroepidemiologic studies have shown convincingly that antibodies to *B. pertussis* antigens can be detected in the population, irrespective of the local immunization schedule, indicating that the circulation of *B. pertussis* in populations is maintained irrespective of current vaccination programmes (see below, *Serosurveys for B. pertussis antibodies*).

**Development of antibodies after primary infection**

The development of pertussis antibodies following disease has been studied by various authors (Aleksandrowicz & Pstragowska, 1980; Nagel & Poot-Scholtens, 1983; Granstrom et al., 1988; Trollfors et al., 1999; Ward et al., 2006, Watanabe et al., 2006). There is a significant rise of IgG and IgA antibodies to PT, FHA and other antigens (Nagel & Poot-Scholtens, 1983; Granstrom et al., 1988). In infants, six to seven weeks is needed for the serum IgA antibody to reach a high level (Nagel & Poot-Scholtens, 1983). As outlined above, PT is the only antigen specific for *B. pertussis*, and antibodies to FHA (Vincent et al., 2000) may be produced, resulting from different antigenic stimulation. However, antibodies to PT are produced only in about 80%–85% of patients after natural infection (Zackrisson et al., 1989 and 1990; Thomas et al., 1989a and 1989b).

**Immune responses after non-primary stimulation**

All populations are more or less continuously exposed to *B. pertussis* antigens, and they maintain a certain level of antibodies to PT and other *B. pertussis* antigens. Reinfections with *B. pertussis* are characterized by a very rapid increase in antibodies making diagnosis more difficult as titre increases may not be seen between acute and convalescent serum samples. As a consequence, titre decreases of more than 50% have been used for defining recent contact with the bacteria (Trollfors et al., 1999). This makes the establishment of cutoffs for IgG-anti-PT in serum samples with recent contact to *B. pertussis* antigens somewhat problematic. Some of the proposed cut-offs are shown in Table 5.

**Transplacental passage of antibodies**

Newborns acquire antibodies passively from their mothers. IgG antibodies against FHA, PT, AGG2 and AGG3 have been detected in cord serum, or in serum from healthy children before their first DwpPT immunization, which represents transplacentally-acquired maternal IgG (Granstrom et al., 1982; Baraff et al., 1984; Celko et al., 1984; Thomas et al., 1989b; Van Savage et al., 1990; Plans et al., 2008; Heininger et al., 2009). The infant’s pertussis IgG antibody level against PT and FHA is comparable to the corresponding maternal level (Van Savage et al., 1990; Healy et al., 2004; Heininger et al., 2009). One study reported that 5% of infants had IgA-anti-PT (Thomas et al., 1989b), although other studies have not substantiated this. No IgM antibodies to pertussis antigens were detected in cord blood (Baraff et al., 1984). Pertussis agglutinins have been found in the cord serum in varying concentrations, and a correlation between high cord blood levels and protection of the infant has been noted (Izurieta et al., 1996). Passively-acquired maternal antibodies fall to a nadir after several months (Baraff et al., 1984; Van Savage et al., 1990) with a half-life of anti-
PT, anti-FHA and agglutinin antibodies estimated to be 36, 40, and 55 days respectively (Van Savage et al., 1990).

Although there is placental passage of pertussis antibodies, most infants do not seem to be protected against clinical disease during the first months of life. The susceptibility of young infants to life-threatening pertussis has been well documented, with a high incidence of pertussis in the first six months of life. Consequently, attempts have been made to protect newborns against pertussis through maternal immunization. Pregnant women were immunized with six doses of unadsorbed wP vaccine, with a total dose of 150 million pertussis organisms. Most of the newborns showed agglutinin and mouse protective antibody titres equal to or greater than their mothers (Cohen & Scadron, 1943). In most of the early studies with unadsorbed vaccine, the total dosage in terms of volume and numbers of organisms was large by today’s standards. Interest in maternal vaccination during pregnancy has recently been renewed. Several studies are underway to study the immunogenicity and safety of aP vaccines in pregnant women, and to monitor the effectiveness in protecting their young infants from pertussis (see below).

Anti-pertussis antibodies have been found in samples of human milk in Nigeria and the United States, but IgG serum antibody levels were higher than breast-milk levels. On the other hand, the mean IgA antibody levels to pertussis (as well as to H. influenzae type b, Streptococcus pneumoniae, and N. meningitidis) were higher in breast milk than in either maternal or infant sera (Kassim et al., 1989). Colostrum samples contained pertussis antibodies, i.e. agglutinins, anti-PT, or anti-FHA, as did samples of human breast milk (Takahashi et al., 2002). Colostrum containing anti-PT antibodies or agglutinins was shown to protect suckling mice from aerosol challenge with B. pertussis, whereas colostrum lacking these antibodies but containing anti-FHA gave little protection (Oda et al., 1985). The significance of breast-milk pertussis antibodies in enhancing infant immunity to pertussis is unknown.

As a consequence of all these findings, maternal vaccination against pertussis has recently been advocated (Edwards, 2003; Mooi & Greef, 2007).

**Duration of protection after natural infection**

Few studies have attempted to determine the duration of protection after B. pertussis infection (Table 6). Symptomatic reinfections are common in adolescents and adults and have recently also been found in children (Broutin et al., 2004). It is therefore difficult to distinguish between the duration of immunity induced by primary infection, and the immunity induced by symptomatic or asymptomatic reinfections. While Gordon and Hood (1951) assumed a near lifelong protection, a cohort study in Germany (Wirsing von Koenig et al., 1995) assumed a protection of 15 years, modeling studies assumed a duration of 7–10 years (Miller & Gay, 1997), and a case series from the Netherlands assumed a protection between three and 12 years (Versteegh et al., 2002). Case reports have been published of symptomatic reinfections as early as 3.5 years after a previous infection (Versteegh et al., 2002).

**Antibody decay after natural infection**

Studies from Germany (Heininger et al., 2004), Japan (Tomoda et al., 1991, the Netherlands (de Melker et al., 2000; Versteegh et al., 2005), and the United States (Hodder et al., 2000), measuring IgG-anti-PT after infection with B. pertussis in populations with high vaccine coverage, have shown that pertussis antibodies quickly increased to peak levels of more than 100 IU/ml, but with great individual variation. Subsequently, antibody levels decreased rapidly, so that after five years all subjects had levels of IgG-anti-PT <10 IU/ml.
A mathematical model used for the Dutch data predicted that, depending on the age of the patient, most of the patients would be below the usual cut-off level of 100 IU/ml after one year (Table 7).

**Serosurveys for *B. pertussis* antibodies**

The prevalence of pertussis antibody in various age groups in the general population depends on the status of pertussis immunization, the extent of exposure to circulating *B. pertussis* organisms, and the methods used to measure them. As antibodies to PT are specific for *B. pertussis*, only these antibodies can be used in serosurveys as an estimate of the circulation of *B. pertussis*.

Serological studies done in Germany (Wirsing von König et al., 1999), in the Netherlands (De Melker et al., 2000) and in the USA (Marchant et al., 1994; Yih et al., 2000; Baughman et al., 2004), evaluated age-specific cut-offs for single sample serological assays. From these studies it emerged that, in adolescents and adults, IgG-anti-PT antibodies from >100–125 EU/ml (= IU/ml) could be used as an indicator of recent pertussis exposure (Table 5). It was also observed that, in most patients, the IgG-anti-PT levels declined rapidly with time (Versteegh et al., 2005; Mertens et al., 2007).

In countries such as Sweden, where vaccination against pertussis was stopped in 1979 and pertussis disease incidence was high for more than decade before the introduction of acellular vaccines, the prevalence of antibodies in children below five years of age increased with age (Zackrisson et al., 1990). Antibody titres increased in older teenagers, so that 90% of young adults had measurable antibody titres (Granstrom et al., 1982). This agrees with the results of studies in the prevaccination era, which showed that a high proportion of children had experienced pertussis infection by the age of 10 (Fine & Clarkson, 1987). In Palermo, Italy, where the coverage rate with DTwP vaccine was very low, the results of a seroepidemiological study suggest a high exposure of children to *B. pertussis*, resulting in increasing rates with age of seroprevalence of IgG anti-PT antibodies. The overall prevalence of these antibodies determined by the ELISA test was 56%; it increased from 24% in one to three year old children, to 67% in 11 to 12 year old children (Stroffolini et al., 1989).

Astonishingly, in vaccinating countries such as the USA, results of seroepidemiological studies showed similar results in older children, adolescents and adults (Cattaneo et al., 1996, Cherry et al., 1995). Another study in the USA using serum samples from a national nutrition survey found that antibodies to PT indicated that *B. pertussis* was widely circulating in the population (Baughman et al., 2004). The American study also indicated that different cohorts of the population could be distinguished, and it was assumed that an IgG-anti-PT level of more that ~100 EU/ml would reflect a recent contact with the bacteria. Across Europe, other serosurveys were performed that resulted in similar findings (ESEN study) (Pebody et al., 2005).

In New Zealand, when measured after vaccination with WP vaccines, the percentage of recipients with ELISA IgG antibody against pertussis toxin also increased with age, from 16% in five year olds to 63% in the 40 to 49 year age group. The percentage of individuals with antibody dropped to 45% in the to 65 year age group (Lau, 1989). In other countries, similar assumptions concerning the circulation of *B. pertussis* were derived from serosurveys (Maixnerova et al., 1979; Stroffolini et al., 1991; Park et al., 2005; Higa et al., 2008; Yildirim et al., 2008).
Immune Responses to Vaccination

Type of immunity induced by whole-cell and acellular vaccines

wP vaccination induces a broad immune response against many bacterial antigens since they are composed of killed entire bacteria. aP vaccines are composed of between one and five purified detoxified antigens. They consequently induce immunity against only a few bacterial proteins involved in the virulence of the bacterium. Thus, vaccine-induced immunity is different, with bacterial virulence factors becoming the major target after immunization with aP vaccines. Given these differences in immune responses, the replacement of wP by aP vaccines should be accompanied by a surveillance of disease to evaluate the consequences of this replacement on herd immunity, and also a surveillance of the bacterial population.

Various clinical case definitions of pertussis based on clinical symptoms and laboratory confirmation have been proposed for vaccine studies and for surveillance purposes (Table 4). The sensitivity and specificity of these clinical case definitions have been evaluated (Blackwelder et al., 1991, Patriarca et al., 1998).

Response to whole-cell vaccine

Vaccination results in an increase in the ELISA antibody titres to a variety of antigens of B. pertussis organisms. Children vaccinated with wP pertussis vaccines may show increasing levels of antibodies against FHA, PT, AGG, LPS and outer membrane protein, depending on the vaccine and immunization schedule (Ashworth et al., 1983; Baraff et al., 1984; Barkin et al., 1984; Halsey & Galazka, 1985; Wilkins et al., 1987; Blumberg et al., 1991; Grimprel et al., 1996).

The extent of the response was proportionate to the number of doses administered. Elevated levels of antibodies to outer membrane protein (OMP) and lipo oligosaccharide (LOS) were also found in sera of unvaccinated children, presumably directed against cross-reacting non-pertussis antigens (Ashworth et al., 1983). Antibody responses to vaccination given immediately after birth have also been reported (Provenzano et al., 1965).

In most studies, more than 70% of children responded to three doses of DTwP vaccine with an agglutinin titre of 1:80 or more. However, wP pertussis vaccines from various manufacturers differ considerably in their immunogenicity. The mean agglutinin titre after three doses of DTwP vaccine ranged between 1:1826 (Barkin et al., 1984) and 1:87 (Blumberg et al., 1991). In a study in France, three doses of DTwP polio vaccine (adsorbed on calcium phosphate) failed to stimulate an agglutinin level of 1:10 in 25% of children, and the mean titre (1:23) was low (Relyveld et al., 1991). A clinical trial conducted at two different academic centres in the USA showed that two commercially available wP vaccines consistently differed in their ability to induce antibody to PT. Infants receiving the Lederle vaccine produced a 46-fold increase in antibody to pertussis toxin, when compared to only a 2.4-fold increase in PT antibody in infants receiving the Connaught vaccine. The FHA and AGG responses to the two wP vaccines were comparable (Edwards et al., 1991b). Antibodies to PT as measured by CHO-cell assay also increased following immunization. Three doses of the wP vaccine caused a moderate response in neutralizing antibody titres (Blennow et al., 1988; Blumberg et al., 1991).

As outlined above, wP vaccines with similar production processes differ in their antigenic dose, and so differences in immunogenicity of different wP vaccines are not surprising.
Levels of antibody against PT, FHA, outer membrane proteins, agglutinins and neutralizing antitoxins, decline considerably during the first year after the completion of a primary series (Barkin et al., 1984; Blennow & Grandstrom, 1989a; Blumberg et al., 1991; Edwards et al., 1991b; Relyveld et al., 1991).

Serological studies provide strong evidence for the booster effect of the fourth dose of DTwP vaccine administered at the end of the second year of life, since antibodies against PT, FHA, and agglutinins increase significantly after the booster dose (Chen et al., 1957; Barkin et al., 1984; Pichichero et al., 1987; Edwards et al., 1991a; Relyveld et al., 1991), but the levels of antibody differ considerably (Barkin et al., 1984; Relyveld et al., 1991).

**Effectiveness of whole-cell vaccine**

The efficacy and effectiveness of wP vaccines has been repetitively shown in vaccine trials (MRC) and in the field. As pointed out before, it must always be kept in mind that wP vaccines are produced by similar methods but may differ significantly in their immunogenicity and their effectiveness. As also mentioned before, significant differences in efficacy were observed in vaccine studies in the 1990s among wP vaccines that had all been cleared by the regulatory agencies using the usual tests for vaccine potency (mouse intracerebral protection) (Edwards & Decker, 2008). Given the array of clinical presentations of pertussis, assessing the effectiveness of wP vaccines is difficult; furthermore the pitfalls of using immunization registry data to determine vaccine effectiveness has recently been highlighted (Mahon et al., 2008).

In many countries, e.g. France, the effectiveness of wP vaccines appeared to remain unchanged at a high level for more than 30 years (Baron et al., 1998). Similarly, in Australia, the effectiveness of the locally produced wP vaccine was estimated to be 0.91 (Torvaldsen et al., 2003). In an outbreak in the USA, the effectiveness of wP vaccine was estimated to be 0.76 (Kenyon et al., 1996). In Poland, it was observed that, for reasons unknown, the effectiveness decreased between 1996 and 2001 from 0.973 to 0.735 (Zielinski et al., 2004). In the Netherlands, the Health Council presented data on the reduced effectiveness of the locally produced wP vaccine and instead recommended the use of aP vaccines (Visser, 2004).

In Austria, the effectiveness of a three-dose course of wP vaccine for the prevention of pertussis hospitalization was estimated to be 0.79 when compared to 0.92 after a three-dose course of aP vaccines (Rendi-Wagner et al., 2006). A similar decrease in hospitalization after changing from wP to aP vaccines was observed in Canada (Bettinger et al., 2007). By contrast, a study in rural Senegal reported that wP vaccines were more effective (0.67) than a two-component aP vaccine (0.32) (Preziosi & Halloran, 2003).

**Effectiveness of incomplete primary series of whole-cell vaccine**

No formal efficacy studies have addressed the effect of an incomplete primary vaccination series or of single doses. Estimates on the effectiveness of incomplete primary series result from surveillance data, and some of these estimates, with their relative endpoints, are given in Table 8.

**Effectiveness of whole-cell vaccine in adolescents and adults**

Few studies have been performed to evaluate the immunogenicity and safety of wP vaccines in adolescents and adults because pertussis was not perceived as a relevant problem in these age groups, and the reactogenicity of wP vaccines was thought to be too high for routine use in older children, adolescents and adults. Nevertheless, between 1933 and 1975,
Immune responses to acellular pertussis vaccines

Due to the use of purified antigens in aP pertussis vaccines, the PT response to primary and booster immunization with aP pertussis vaccine is usually more pronounced than the response to wP vaccine (Pichichero et al., 1987; Anderson et al., 1987 Morgan et al., 1990; Van Savage et al., 1990; Edwards et al., 1995). Compared with wP vaccine, significantly higher anti-PT and anti-FHA responses have been reported with aP vaccines containing these antigens (Edwards & Decker, 2008). Differences have also been found between responses to aP and wP vaccines in infants with various preimmunization levels of IgG ELISA antibody to PT. The response to aP vaccine was independent of the preimmunization antibody titre, while the response to wP vaccine was strongly dependent on the preimmunization titre. It is not known whether the better response to aP vaccine among those with higher anti-PT titres was due to greater immunogenicity of PT in the aP product, the absence of some component of the wP vaccine, or other, as yet, unidentified factors (Van Savage et al., 1990). IgG anti-PT levels do not differ between natural infection or vaccination (Giammanco et al., 2003).

The diphtheria and tetanus responses in children receiving aP- and wP-pertussis component DPT vaccines were similar in some studies (Pichichero et al., 1987; Anderson et al., 1987 Edwards et al., 1991a) and lower in the NIH trial (Edwards et al., 1995). The efficacy studies in Europe and Africa showed no relevant differences in the diphtheria and tetanus response between the DTwP and DTaP recipients (Edwards & Decker, 2008).

Effectiveness of acellular vaccines in infants

After the successful introduction of acellular vaccines in Japan (Aoyama et al., 1988), a number of large vaccine efficacy studies with aP vaccines have been performed in Africa and Europe. Although these studies used different designs, were performed in different populations, and employed different vaccines, they all used the WHO case definition for pertussis and so the results of the studies have been repetitively compared and summarized (Edwards & Decker, 2008). An example of the various estimates of efficacy is given in table 2. Meanwhile, effectiveness estimates are available due to the broad use of aP vaccines in the population, (Elliott et al., 2004; Edwards & Decker, 2008). Although the isolates of B. pertussis have undergone some changes in their genomic makeup and also in the expression of virulence factors such as PRN when compared to the Tohama strain, used for the production of all aP vaccines (He et al., 2003; Hallander et al., 2007), no significant changes in the effectiveness of aP vaccines have been observed over time.

Effectiveness of incomplete primary series of acellular vaccines

As expected, no formal efficacy study has addressed the effect of an incomplete primary vaccination or of single doses. However, during the aP vaccine efficacy studies, some estimates of the effectiveness of incomplete series were performed. During a prolonged enhanced surveillance in Sweden following the efficacy trials there, the rates of pertussis were 225/100 000 in unvaccinated infants aged 0–2 months, 212/100 000 after one dose in infants aged 3–4 months, 31/100 000 after two doses in infants aged 5–11 months and 19/100 000 after three doses (Gustaffson et al., 2005). A hospital-based survey in Germany estimated that even one dose of vaccine was 68% effective in reducing hospitalization in infants (Juretzko et al., 2002). A case-control study in the USA (Bisgard et al., 2005) found that the effectiveness of one dose of acellular vaccine was approximately 50%. Results of the studies are summarized in Table 8.
Effectiveness of acellular vaccines in adolescents and adults

During the last two decades, many reports have shown an increase of pertussis in adolescents and adults (Wirsing von König et al., 2002 Halperin, 2007). This may reflect a shift in the age distribution of pertussis, since the disease is effectively controlled by vaccine among children. On the other hand, several authors have expressed concern that pertussis immunity may be only partial among adults (Fine & Clarkson, 1987). The decreased immunity among adults may be related to the reduced circulation of pertussis organisms in well-vaccinated populations, with subsequent less frequent exposure to \textit{B. pertussis} and less natural boosting. The increase in cases among adolescents and adults may also be due to an increased awareness of the disease in adolescence and adulthood. Finally it could be the effect of more sensitive laboratory methods (i.e. serology) to detect the infection (Cagney et al., 2008).

As a consequence, the importance of late booster doses of \textit{aP} vaccines for maintaining immunity against pertussis in older children or adolescents has been discussed intensively and many countries have now recommended booster doses in adolescents and adults. A vaccine efficacy study conducted in the USA obtained a point estimate of vaccine efficacy in adolescents and adults of 0.92 (Ward et al., 2005). Another effectiveness study of an adolescent booster dose conducted in Australia had a point estimate of 0.78 (Rank et al., 2009). Given the intensive circulation of the bacteria, even one dose of an \textit{aP} vaccine given to adolescents without a history of pertussis disease or vaccination, induced an immune response in nearly all vaccinees (Knuf et al., 2006).

Many countries in the European Union, as well as Australia, Canada and the United States, recommend a preschool and/or an adolescent booster. The USA and many other countries have also introduced a booster for the adult population using a Tdap combination vaccine (CDC, 2006; CDC, 2009).

Correlates of protection for pertussis vaccines

No serological correlate for protection after vaccination with \textit{wP} vaccines has been established, although the MRC trial already suggested a correlation between high agglutinin titres and protection (Table 9).

Various studies have attempted to find a serological correlate of protection after vaccination with \textit{aP} vaccines (Table 9). It could be shown, using data derived from the Swedish NIH-sponsored trial (Storsaeter et al., 1998), that subjects with detectable IgG-anti-PT but with non-detectable anti-PRN and anti-FIM had an assumed vaccine efficacy of 46%. Those with anti-PT and anti-FIM had an estimated efficacy of 72%, those with anti-PT and anti-PRN had an assumed vaccine efficacy of 75%, and those with all three antibodies had an assumed vaccine efficacy of 85%. A German study (Cherry et al., 1998) used thresholds for antibodies and found that subjects with anti-PRN titres were best protected, that high anti-PT contributed to protection, but that anti-FHA and anti-FIM did not correlate with protection. In a Finnish study, IgG-anti-FHA at elevated levels correlated best with protection (He et al., 1994). Following the cohort of the Swedish vaccine study, it was assumed that low or undetectable levels of IgG-anti-PT would be the best predictor of susceptibility to reinfection (Storsaeter et al., 2003). The Gothenburg study, using a PT-only vaccine (Taranger et al., 2000) showed that the induction of anti-PT induced good protection.

No correlate of protection for cell-mediated immunity against the different pertussis antigens has been observed so far.
Overall, it seems most probable that no single correlate of protection does exist, and that antibodies to many antigens in differing amounts, probably in cooperation with cell-mediated immunity, confer protection against symptomatic reinfection.

**Interchangeability of pertussis vaccines**

Few studies have addressed the interchangeability of aP or wP pertussis vaccines from different manufacturers during primary vaccination. It seems clearly advisable not to interchange wP or aP vaccines from different manufacturers during the primary series.

Tripedia® (Sanofi) and Infanrix® (GSK) were interchanged in one study (Greenberg et al., 2002) and no differences in immunogenicity were observed. Similar results were found in Canada, when Pentacel® (Sanofi) and Infanrix® (GSK) were interchanged during primary immunization (Halperin et al., 2006). Another study interchanging Acelimune® (Wyeth) and Tripedia® (Sanofi) again produced non-significant differences in immunogenicity (Wirsing von König et al., 2000). The Canadian Immunization Guide stated that for primary immunization, a vaccine from the same manufacturer should be used whenever possible. For the 18-month booster, and for the preschool booster, experts agreed that aP containing combination vaccines can be interchanged without loss in immunogenicity (NACI, 2005; Canadian Immunization Guide 2006).

**Duration of protection after vaccination with whole-cell vaccines**

Many studies have provided strong evidence that the wP pertussis vaccines are effective in protecting the individual against typical pertussis, either by preventing its occurrence altogether or by markedly reducing its severity (Griffiths, 1988; Wendelboe et al., 2005). However, the duration of immunity following pertussis vaccination is still an open issue, and it may also depend on the intervals used during primary vaccination (Silfverdal et al., 2007). This is reflected in differences in the immunization schedules used. Usually, the primary series consists of three doses of DTwP vaccine given during the first year of life. In the WHO African and South-East Asia Regions, most countries use the immunization schedule recommended by the Expanded Programme on Immunization (EPI), which calls for three doses of DTwP vaccine at 6, 10 and 14 weeks; however some countries use a 3, 4 and 5 month schedule (WHO, 2009). In the Region of the Americas, the schedule used in the United States, with two- month intervals between doses in the primary series, is mostly followed (WHO).

It is noteworthy that for 14% to 18% of countries in the Americas, European, Eastern Mediterranean and Western Pacific Regions, the third dose of DTwP vaccine is recommended at a late age, generally after six months of age, so this may reflect a two-dose primary immunization with a booster. Various immunization schedules used by countries in the European Region are available on the EUVACNET website (EUVACNET, 2009). The WHO website contains information about schedules and coverage rates worldwide (WHO, 2009).

Epidemiological observations suggest that the efficacy of pertussis vaccine is high only for a limited period of time, and falls gradually with time after immunization. In the United Kingdom, the vaccine efficacy fell from 100% in the first year following three doses of DTwP vaccine to 46% in the seventh year (Jenkinson, 1988). In another outbreak study in the USA it was estimated that protection lasted about 12 years after wP vaccination. In Sweden, the efficacy of three doses of unadsorbed wP pertussis vaccine declined from 89% in 6–11 month old children to 76% in children at the end of the second year of life (Blennow et al., 1988). Other cohort and case-control studies suggest a slight but continuous decrease in
vaccine efficacy with time (Fine & Clarkson, 1987). Various studies from surveillance data assume an estimate of protection between five and 14 years, and these studies are summarized in Table 10.

About one-third of countries in the Americas and Western Pacific Regions give a fifth dose of DTwP vaccine ("second booster"). The need for a fifth dose of DTwP vaccine and its importance in controlling pertussis in other regions remains to be proven. Serologically, this additional dose of DTwP vaccine seems to exert a clear booster effect (Edwards et al., 1991a Morgan et al., 1990).

Antibody decay after immunization with whole-cell vaccines

Few studies have addressed the antibody decay after vaccination with wP vaccines (Grimprel et al., 1996). Overall, the relatively low levels of antibodies induced by wP vaccines decline rapidly below detection levels one to two years after vaccination (Blennow & Granström, 1990).

Duration of protection after vaccination with acellular vaccines

After completion of the aP vaccine studies in the 1990s, their long-time effectiveness was closely monitored. Studies in Italy (Salmaso et al., 2001) showed that efficacy remained almost unchanged until six years of age. Gustafsson et al. (2005) showed that protection began to wane in 7–8 year olds. These findings were corroborated by many other studies, indicating an interval of unchanged protective effectiveness of between 5.5 and 7 years (Germany, Senegal, Sweden) (Table 10). Thus, it has been suggested that a school-entry vaccination is necessary after a three-dose primary course of immunization (Carlsson & Trollfors, 2009). Some countries (i.e. Australia), have even skipped the first booster in the second year of life in favour of a preschool booster. As mentioned above, it has to be kept in mind that pertussis is not the only vaccine component, and that apart from the effectiveness of the vaccine, implementation issues concerning vaccine coverage may play an important role.

It is as yet unclear how long the protection after vaccination with Tdap vaccines in adolescents and adults will last.

Antibody decay after immunization with acellular vaccines

Primary immunization: The aP vaccine trial in Italy and Sweden also produced data about the antibody decay in the study populations. Giulinoa et al. (1998) showed that 15 months after immunization with three doses of aP vaccines, antibodies to PT, FHA and pertactin had mostly decreased below the level of detection, irrespective of a sustained vaccine efficacy (Salmaso et al., 2001).

Booster immunizations: Tdap boosters result in rapid responses to pertussis antigens in adults (Kirkland et al., 2009). The APERT study (Le et al., 2004) suggested that these antibodies to PT will be above the level of detection for about five years. Other recent studies into the decay of antibodies after booster vaccination in adolescents and adults assume that after a steep decline in the first year after vaccination antibodies decline gradually, and may be detectable longer than five years after aP administration. In this study, after one month, a geometric mean titre (GMT) of 38 EU/ml of IgG-anti-PT was found and after one year, mean IgG-anti-PT levels had decreased to 8 EU/ml. McIntyre et al. (2004) found a peak GMT of 83 EU/ml IgG-anti-PT that decreased to 30 EU/ml after one year. Edelman et al. (2004 and 2007), using the same vaccine in adolescents, found a peak GMT of IgG-anti-PT of 116 EU/ml four weeks after vaccination, which decreased to 16 EU/ml after three years and to 8 EU/ml five years after vaccination. Riffelmann et al. (2009)
vaccinated health-care workers and found a higher peak value, with a rapid decline in antibody over the first year after vaccination and a slower decrease in the three consecutive years (Table 11). Modelling the decay of antibodies after vaccination suggested Tdap booster doses every 10 years would be necessary (Bailleux et al., 2008).

Summary

*Bordetella pertussis* produces an array of virulence factors that act together inducing the clinical symptoms of pertussis or whooping cough. The human immune response to antigens of *B.pertussis* can be measured by ELISA, by bacterial agglutination, and by various other tests, including those measuring cell-mediated immunity. Different pertussis vaccines are available and licensed, consisting of either whole bacterial cells (whole-cell = wP-vaccines) or of purified virulence factors (acellular = aP-vaccines). Both types of vaccines have been shown to be effective, but aP vaccines show less local and systemic side-effects, and they can be produced more reproducibly. Both wP and aP antigens are mostly combined with tetanus and diphtheria toxoid to give DTwP and DTaP vaccines. Combination vaccines, with the addition of other antigens, are also licensed and widely used. Neither infection nor vaccination induces a life-long immunity, reinfections are frequent, and *B.pertussis* circulates all over the world. The amount of circulation can be estimated by serosurveys. In order to maintain protection against pertussis throughout life, infant immunization schedules are being extended in many countries to include pre-school boosters, and also vaccination of adolescents and adults.
Figure 1

Diagrammatic sketch of some *B. pertussis* virulence factors

(Adapted, by permission of Dr. Heinz-Josef Schmitt)
References


EUVACNET. Pertussis vaccination overview in European countries (http://www.euvac.net/graphics/euvac/vaccination/pertussis.html).


Hallander H et al. (2005). Pertussis antitoxin decay after vaccination with DTaP. Response to a first booster dose $\frac{3}{2}$–$\frac{6}{2}$ years after the third vaccine dose. *Vaccine*, 23:5359–5364.


Koesters K et al. (2000). Comparison of five commercial enzyme-linked immunosorbent assays for detection of antibodies to *Bordetella pertussis*. *Clinical and Diagnostic Laboratory Immunology*, 7:422–426.


Maixnerova M et al. (1979). Immunological surveys of antibodies against *B. pertussis* and *B. parapertussis* in some African and Asian countries. *Journal of Hygiene, Epidemiology, Microbiology, and Immunology*, 23:201–211.


Stehr K et al. (1998). A comparative efficacy trial in Germany in infants who received either the Lederle/Takeda acellular pertussis component DTP (DtaP) vaccine, the Lederle whole-cell component DTP vaccine, or DT vaccine. *Pediatrics*, 101:1–11.


## Table 1

**Virulence factors of B. pertussis, polymorphisms, isotypes of antibodies**

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th>Function / structure</th>
<th>Vaccine component</th>
<th>Antibody isotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHA</td>
<td>Adhesion</td>
<td>wP, most aP</td>
<td>IgG, IgA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface associated + secreted</td>
<td></td>
</tr>
<tr>
<td>Fimbriae</td>
<td>Adhesion</td>
<td>wP, many aP</td>
<td>IgG, IgA, IgM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface associated</td>
<td></td>
</tr>
<tr>
<td>Pertactin</td>
<td>Cell binding, adhesion</td>
<td>wP, many aP</td>
<td>IgG, IgA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface protein</td>
<td></td>
</tr>
<tr>
<td>BrkA</td>
<td>Possible adhesion</td>
<td>wP</td>
<td>IgG, ?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface protein</td>
<td></td>
</tr>
<tr>
<td>PT</td>
<td>Suppression of host response</td>
<td>wP, all aP</td>
<td>IgG, IgA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adhesion</td>
<td></td>
</tr>
<tr>
<td>ACT</td>
<td>Suppression of host response</td>
<td>wP</td>
<td>IgG, ?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface associated and secreted</td>
<td></td>
</tr>
<tr>
<td>Dermonecrotic toxin</td>
<td>Induction of host cell necrosis</td>
<td>wP</td>
<td>IgG, ?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Secreted</td>
<td></td>
</tr>
<tr>
<td>Tracheal cytotoxin</td>
<td>Damage to cilia</td>
<td>wP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peptidoglycan-byproduct</td>
<td></td>
</tr>
<tr>
<td>LOS/LPS</td>
<td>Induces fever + other LPS responses</td>
<td>wP</td>
<td>IgM, IgG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gram-negative cell surface</td>
<td></td>
</tr>
</tbody>
</table>
Table 2
Composition of some acellular vaccines and efficacy estimates (Edwards & Decker, 2008)

<table>
<thead>
<tr>
<th>Acellular vaccine</th>
<th>Composition</th>
<th>Producer</th>
<th>Study site</th>
<th>% VE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acelluvax</td>
<td>5 µ PT, 2.5 µg FHA, 2.5 µg PRN, 25 Lf D, 10 Lf T</td>
<td>Chiron, now Novartis</td>
<td>Italy</td>
<td>84% (76–90%)</td>
</tr>
<tr>
<td>Tripedia</td>
<td>23.4 µg PT, 23.4 µg FHA, 6.7 Lf D, 5 Lf T</td>
<td>Aventis Pasteur, now Sanofi</td>
<td>Germany</td>
<td>93% (63–99%)</td>
</tr>
<tr>
<td>Infanrix</td>
<td>25 µg PT, 25 mg FHA, 8 µg PRN, 25 Lf D, 10 Lf T</td>
<td>GlaxoSmithKline Biologicals</td>
<td>Italy</td>
<td>84% (76–89%)</td>
</tr>
<tr>
<td>Infanrix</td>
<td>25 µg PT, 25 mg FHA, 8 µg PRN, 25 Lf D, 10 Lf T</td>
<td>GlaxoSmithKline Biologicals</td>
<td>Germany</td>
<td>89% (77–95%)</td>
</tr>
<tr>
<td>Tripacel</td>
<td>10 µg PT, 5 µg FHA, 3 µ PRN, 3 µg FIM, 15 Lf D, 6 Lf T</td>
<td>Aventis Pasteur, now Sanofi</td>
<td>Sweden</td>
<td>85% (81–89%)</td>
</tr>
<tr>
<td>Certiva</td>
<td>40 µg PT, 15 Lf D, 6 Lf T</td>
<td>Baxter</td>
<td>Sweden</td>
<td>71% (63–78%)</td>
</tr>
<tr>
<td>Acel-Immune</td>
<td>3.5 µg PT, 35 mg FHA, 2 µg PRN, 0.8 µg FIM, 9 Lf D, 5 Lf T</td>
<td>Wyeth</td>
<td>Germany</td>
<td>78% (60–88%)</td>
</tr>
</tbody>
</table>

VE: Vaccine efficacy
Table 3  
Methods for detection of antibodies

<table>
<thead>
<tr>
<th>Method</th>
<th>Quantification</th>
<th>Antigens</th>
<th>Isotypes</th>
<th>Reported unit</th>
<th>Standardized</th>
<th>Commercially available</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>Yes</td>
<td>PT, FHA, PRN, FIM</td>
<td>IgG, IgA</td>
<td>IU/ml</td>
<td>Partly</td>
<td>Yes</td>
</tr>
<tr>
<td>Immunoblot</td>
<td>Semiquantitative</td>
<td>PT, FHA, ACT</td>
<td>IgG, IgA</td>
<td>n.a.</td>
<td>partly</td>
<td>Yes</td>
</tr>
<tr>
<td>Agglutination</td>
<td>Semiquantitative</td>
<td>Whole cells</td>
<td>IgM (IgG)</td>
<td>Titres</td>
<td>Partly</td>
<td>No</td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>Yes</td>
<td>PT, FHA, PRN</td>
<td>IgG, IgA</td>
<td>IU/ml</td>
<td>Partly</td>
<td>Yes</td>
</tr>
<tr>
<td>CHO-cell assay</td>
<td>Semiquantitative</td>
<td>PT</td>
<td>IgG</td>
<td>Titres</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>IFT</td>
<td>Semiquantitative</td>
<td>Whole cells</td>
<td>?</td>
<td>Titres</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

CHO, Chinese hamster ovary.  
IFT, Indirect immunofluorescence test  
n.a. = not applicable.
<table>
<thead>
<tr>
<th>Clinical symptoms</th>
<th>Duration</th>
<th>Confirmation</th>
<th>Intended use</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paroxysmal cough</td>
<td>&lt;21 days</td>
<td>Positive culture</td>
<td>Efficacy trials</td>
<td>WHO, 1991</td>
</tr>
<tr>
<td>+ Increase of IgG/IgA anti-PT / FHA / AGG2/3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Contact with culture proven case</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>&lt;14 days</td>
<td>Positive culture</td>
<td>Surveillance</td>
<td>CDC, 1997</td>
</tr>
<tr>
<td>+ Paroxysms and/or</td>
<td></td>
<td>Positive PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ whoop and/or</td>
<td></td>
<td>Contact with proven case</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ post-tussive vomiting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pertussis diagnosed by a physician</td>
<td></td>
<td>Positive culture</td>
<td>Surveillance</td>
<td>WHO, 2000</td>
</tr>
<tr>
<td>Cough</td>
<td>&lt;14 days</td>
<td>Positive PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ paroxysms and/or</td>
<td></td>
<td>Positive paired serology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ whoops and/or</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ post-tussive vomiting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PCR = polymerase chain reaction
Table 5

Proposed cut-off values of IgG-anti-PT for diagnostic serology to indicate recent contact

<table>
<thead>
<tr>
<th>Country</th>
<th>Type of study</th>
<th>Cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA, USA</td>
<td>Population study</td>
<td>~ 100 IU/ml</td>
<td>78%</td>
<td>98%</td>
<td>Marchant et al., 1994</td>
</tr>
<tr>
<td>MA, USA</td>
<td>Population study</td>
<td>~200 IU/ml</td>
<td>67%</td>
<td>99.9%</td>
<td>Yih et al., 2000</td>
</tr>
<tr>
<td>NL</td>
<td>Population study</td>
<td>125 IU/ml</td>
<td>70%</td>
<td>99%</td>
<td>de Melker et al., 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>62 IU/ml</td>
<td>80%</td>
<td>95%</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Population study</td>
<td>50 IU/ml</td>
<td>80%</td>
<td>95%</td>
<td>Wirsing von König et al., 1999</td>
</tr>
<tr>
<td>EU</td>
<td>Epid. survey</td>
<td>125 IU/ml</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Pebody et al., 2005</td>
</tr>
<tr>
<td>USA</td>
<td>Epid. survey, model</td>
<td>94 IU/ml</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Baughman et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>46 IU/ml</td>
<td>n.a.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUS</td>
<td>Clinical validation</td>
<td>50 IU/ml</td>
<td>better than 100 IU/ml</td>
<td></td>
<td>Horby et al., 2005</td>
</tr>
</tbody>
</table>

MA, Massachusetts.
n.a., not available.
Table 6
Duration of protection after natural infection

<table>
<thead>
<tr>
<th>Year</th>
<th>Type of study</th>
<th>Estimated years of protection</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1951</td>
<td>Review</td>
<td>Lifelong</td>
<td>Mainly United Kingdom</td>
<td>Gordon &amp; Hood, 1951</td>
</tr>
<tr>
<td>1995</td>
<td>Household contact</td>
<td>15–20</td>
<td>Germany</td>
<td>Wirsing von Koenig et al., 1995</td>
</tr>
<tr>
<td>2002</td>
<td>Case series</td>
<td>3.5–12</td>
<td>Netherlands</td>
<td>Versteegh et al., 2002</td>
</tr>
</tbody>
</table>
Table 7
Antibody decay after natural infection compared with peak values

<table>
<thead>
<tr>
<th>Year</th>
<th>Type of study</th>
<th>Antibodies</th>
<th>Decay after one year</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>Cohort study</td>
<td>IgG-anti-PT</td>
<td>~55%</td>
<td>USA</td>
<td>Hodder et al., 2000</td>
</tr>
<tr>
<td>2000</td>
<td>Population survey</td>
<td>IgG-anti-PT</td>
<td>~80%</td>
<td>NL</td>
<td>de Melker et al., 2000</td>
</tr>
<tr>
<td>2000</td>
<td>Cohort study</td>
<td>IgG-anti-PT</td>
<td>~66%</td>
<td>D</td>
<td>Heininger et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgA-anti-PT</td>
<td>~70%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 8
Estimated degree of protection achieved after different doses of pertussis vaccine

<table>
<thead>
<tr>
<th>Year/type of study</th>
<th>Vaccine</th>
<th>Dose</th>
<th>End-point</th>
<th>VE</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990 /surveillance</td>
<td>wP</td>
<td>1</td>
<td>Pertussis</td>
<td>0.44</td>
<td>USA</td>
<td>Onorato et al., 1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>&quot;</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2004 /case-control</td>
<td>Mostly aP</td>
<td>1</td>
<td>Pertussis (CDC)</td>
<td>0.505</td>
<td>USA</td>
<td>Bisgard et al., 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>&quot;</td>
<td>0.801</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>&quot;</td>
<td>0.933</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1996–1998/surveillance</td>
<td>Mostly aP</td>
<td>1</td>
<td>Hospitalization</td>
<td>0.680</td>
<td>Germany</td>
<td>Juretzko et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>&quot;</td>
<td>0.918</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>&quot;</td>
<td>0.998</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1990–/surveillance</td>
<td>wP</td>
<td>1</td>
<td>Hospitalization</td>
<td>0.36</td>
<td>Denmark</td>
<td>Hviid, 2009</td>
</tr>
<tr>
<td>2004</td>
<td></td>
<td>2</td>
<td>&quot;</td>
<td>0.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>&quot;</td>
<td>0.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1990–/surveillance</td>
<td>ap</td>
<td>1</td>
<td>&quot;</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td></td>
<td>2</td>
<td>&quot;</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>&quot;</td>
<td>0.96</td>
<td></td>
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Table 9
Suggested correlates of protection after vaccination with acellular vaccines

<table>
<thead>
<tr>
<th>Antibody type</th>
<th>Study type</th>
<th>Vaccine type</th>
<th>Correlation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agglutinins (anti-FIM)</td>
<td>Vaccine trial</td>
<td>wP</td>
<td>High titres protect</td>
<td>Medical Research Council, 1959</td>
</tr>
<tr>
<td>Agglutinins (anti-FIM)</td>
<td>Household contact</td>
<td>wp (?)</td>
<td>High titres protect</td>
<td>Deen et al., 1995</td>
</tr>
<tr>
<td>Anti-PRN</td>
<td>Household contact</td>
<td>wp (?)</td>
<td>High titres protect</td>
<td>Deen et al., 1995</td>
</tr>
<tr>
<td>Anti-PRN</td>
<td>Vaccine trial</td>
<td>aP</td>
<td>High titres protect</td>
<td>Storsaeter et al., 1998</td>
</tr>
<tr>
<td>Anti-PRN</td>
<td>Vaccine trial</td>
<td>aP</td>
<td>High titres protect</td>
<td>Cherry et al., 1998</td>
</tr>
<tr>
<td>Anti-PT</td>
<td>Household contact</td>
<td>aP</td>
<td>Low titres make susceptible</td>
<td>Storsaeter et al., 2003</td>
</tr>
<tr>
<td>Anti-PT</td>
<td>Household study</td>
<td>aP</td>
<td>High titres protect</td>
<td>Taranger et al., 2000</td>
</tr>
<tr>
<td>Anti-FIM</td>
<td>Household contact</td>
<td>aP</td>
<td>High titres protect</td>
<td>Storsaeter et al., 1998</td>
</tr>
<tr>
<td>Anti-FHA</td>
<td>Cohort study</td>
<td>wP</td>
<td>High titres protect</td>
<td>He et al., 1996a</td>
</tr>
</tbody>
</table>
Table 10  
Estimated duration of protection after vaccination (three doses)

<table>
<thead>
<tr>
<th>Year</th>
<th>Vaccine</th>
<th>Type of study</th>
<th>Duration</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>wP</td>
<td>surveillance</td>
<td>8 years</td>
<td>United Kingdom</td>
<td>Jenkinson et al., 1988</td>
</tr>
<tr>
<td>1996</td>
<td>wP</td>
<td>Surveillance</td>
<td>5–10</td>
<td>SF / CH</td>
<td>He et al., 1996b</td>
</tr>
<tr>
<td>1999</td>
<td>wP</td>
<td>Surveillance</td>
<td>5–14</td>
<td>United Kingdom</td>
<td>van Buynier et al., 1999</td>
</tr>
<tr>
<td>2003</td>
<td>wP</td>
<td>Surveillance</td>
<td>6–9</td>
<td>AUS</td>
<td>Torvaldsen et al., 2003</td>
</tr>
<tr>
<td>2001</td>
<td>aP</td>
<td>Vaccine study</td>
<td>6</td>
<td>I</td>
<td>Salmaso et al., 2001</td>
</tr>
<tr>
<td>2002</td>
<td>aP</td>
<td>Cohort study</td>
<td>6</td>
<td>D</td>
<td>Lugauer et al., 2002</td>
</tr>
<tr>
<td>2006</td>
<td>aP</td>
<td>Vaccine study</td>
<td>6</td>
<td>S</td>
<td>Tindberg et al., 1999</td>
</tr>
</tbody>
</table>
Table 11
Antibody decay after vaccination with acellular pertussis vaccines
Percentage of IgG-anti-PT left from peak value (adapted from Riffelmann et al., 2009)

<table>
<thead>
<tr>
<th>Type of aP vaccine</th>
<th>% left</th>
<th>12 months</th>
<th>18 months</th>
<th>36 months</th>
<th>48 months</th>
<th>60 months</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tdap</strong>: 2.5µg PT, 5µg FHA, 3µg PRN, 5µg FIM, d, T</td>
<td>35%</td>
<td>30%</td>
<td>18%</td>
<td></td>
<td></td>
<td></td>
<td>Barreto et al., 2007</td>
</tr>
<tr>
<td><strong>Tdap</strong>: 8µg PT, 8µg FHA, 2.5µg PRN, d, T</td>
<td>36%</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
<td>McIntyre et al., 2004</td>
</tr>
<tr>
<td><strong>Tdap</strong>: 8µg PT, 8µg FHA, 2.5µg PRN, d, T</td>
<td>n.d.</td>
<td>n.d.</td>
<td>14% (11%–16%)</td>
<td>10% (9%–11%)</td>
<td></td>
<td></td>
<td>Edelman et al., 2004 and 2007</td>
</tr>
<tr>
<td><strong>Tdap</strong>: 8µg PT, 8µg FHA, 2.5µg PRN, d, T</td>
<td>27% (23%–31%)</td>
<td>27% (23%–31%)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
<td>Le et al., 2004</td>
</tr>
<tr>
<td><strong>aP</strong>: 25µg PT, 25µg FHA, PRN traces</td>
<td>29% (4%–114%)</td>
<td>n.d.</td>
<td>19% (3%–77%)</td>
<td>20% (4%–118%)</td>
<td></td>
<td></td>
<td>Riffelmann et al., 2009</td>
</tr>
</tbody>
</table>

n.d., not determined.