The immunological basis for immunization series

Module 4: Pertussis
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## Contents:

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pertussis</td>
<td>5</td>
</tr>
<tr>
<td>Antigens of <em>Bordetella pertussis</em></td>
<td>5</td>
</tr>
<tr>
<td>Pertussis Vaccines</td>
<td>8</td>
</tr>
<tr>
<td>Acellular Pertussis Vaccines</td>
<td>9</td>
</tr>
<tr>
<td>Combination vaccines</td>
<td>10</td>
</tr>
<tr>
<td>Measuring the Immune Response to <em>B. pertussis</em> Antigens</td>
<td>12</td>
</tr>
<tr>
<td>Immune Responses after Exposure to <em>Bordetella pertussis</em></td>
<td>14</td>
</tr>
<tr>
<td>Duration of protection after natural infection</td>
<td>16</td>
</tr>
<tr>
<td>Antibody decay after natural infection</td>
<td>16</td>
</tr>
<tr>
<td>Serosurveys for <em>B. pertussis</em> antibodies</td>
<td>16</td>
</tr>
<tr>
<td>Immune Responses to Vaccination</td>
<td>17</td>
</tr>
<tr>
<td>Summary</td>
<td>24</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>24</td>
</tr>
</tbody>
</table>
PREFACE

This module is part of the Series, “The Immunological Basis for Immunization”, which was initially developed in 1993 as a series of eight modules drawing on the experience of persons working within the World Health Organization’s Expanded Programme on Immunization (EPI). This programme was established in 1974 with the main aim of providing immunization for children in developing countries. Beyond a general immunology module, each of the seven other modules covered one of the vaccines recommended as part of the EPI programme, i.e. diphtheria, measles, pertussis, polio, tetanus, and tuberculosis. The modules became some of the most widely used documents in the field of immunization.

In the context of the Global Immunization Vision and Strategy (GIVS) (web) and the expanded focus of WHO and immunization programmes in general in terms of target populations and antigens, and also to remain relevant in the light of the large accumulation of new knowledge and new vaccine developments since 1993, the decision has been taken to update the series and also to extend it by adding new modules.

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 the immunological basis for the WHO recommendations on vaccine use that appear in the Vaccine Position Papers (web). The general immunology module will not be updated however, and this is because of the constant evolution taking place in the field of immunology, and also the several excellent general textbooks on the subject. General information will in future be given in the form of useful references which will be provided on the WHO web site.

Since 1998, WHO has been issuing a series of regularly updated position papers on vaccines, and vaccine combinations against diseases that carry an international public health impact. These papers, which are concerned primarily with the use of vaccines in large-scale immunization programmes, summarize essential background information on the respective diseases and vaccines, and conclude with the current WHO position concerning their use in the global context. Since April 2006, these have been reviewed and endorsed by WHO’s Immunization Strategic Advisory Group of Experts (SAGE), and published in the WHO Weekly Epidemiological Report. As such, they are limited in their length and the amount of background information which they provide.

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.
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; re-infections are common and occur throughout ones 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* big parts of the genome of *B. pertussis* and *B. parapertussis* were inactivated or lost during adaptation to the human host. As 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 and 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 hemagglutinin, 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 which 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 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-ribosyltransferase and ribosylates G proteins (Pittman 1979, Burns, 1988, Kerr and 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, ptx 1, ptx 2 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 calmodulin activated 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 be also produced in low titers 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. In contrast to other Bordetella spp., the B. pertussis LPS lacks a long O-antigenic chain, and is also called lipooligosaccharide (LOS). LOS is likely responsible for some of the adverse reactions in children following wP pertussis immunization and it has antigenic (although not protective) and adjuvant properties. The amount of LOS in wP vaccines has been shown to be significantly associated with the frequency of fever after vaccination (Baraff et al. 1989). LOS was also recognized one of the agglutinogens, formerly called AGG1. B. pertussis LOS is generally not contained in aP vaccines, depending on the purification processes of aP vaccines.

**Dermonecrotic toxin (DNT, Heat-labile toxin: HLT)**

DNT, one of the first discovered virulence factors of *B. pertussis*, induces dermal necrosis in mice when injected intradermally. This heat-labile toxin 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 (TCT)**

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 (Goldman et al., 1999). TCT is not contained in aP vaccines.

**Filamentous hemagglutinin**

Filamentous hemagglutinin (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 Serin-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 (cf Mattoo and Cherry, 2005). Genomic studies of the *fha*-genes have shown almost no heterogeneity among different strains (Mooi et al., 2007).

FHA is contained in most aP vaccines.

**Pertactin**

Pertactin (PRN), an autotransporters, is a 68-70 kDa surface protein that mediates eukaryotic cell binding by its Arg-Gly-Asp (RGD) motif, and it 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 et al., 2007). Changes in *prn* types have been suspected by some workers to contribute to a reduced vaccine efficacy in whole-cell vaccines in the Netherlands (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. In contrast to antibodies produced in rabbits, PRN antibodies to those epitopes that are influenced by polymorphisms were not 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, 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 as to their role in human pertussis. Antibodies to TcfA slowly develop after primary infection, but rapid increases are noted on secondary exposure. These autotransporters are not contained in aP vaccines.

**Fimbriae**

Fimbriae 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 whol-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 that the FIM type of circulating strains could change over time (Mooi et al., 2007). Whether or not the changes in FIM2 and FIM3 types are induced by vaccination pressure is still under debate. 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.

**BrkA**

BrkA (Bordetella resistance to killing genetic locus, frame A), a 73 kDa protein with a large 30kDa outer surface domain, protects the bacteria against being killed by the classical pathway of complement. Antibodies to BrkA slowly develop 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 therefore may 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 of the world, 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 aluminum 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). Although this test has been used for long time, it is not clear what kind of the murine immune responses 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 5 to 6 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 efficacy of wP vaccines from different
producers. 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 efficacy estimates (VE) of 98% and 96% in two studies. One US 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. In contrast, an US whole-cell vaccine that had passed the potency tests was found to be only 36% efficacious in Italy and 48% in Sweden.

Similar low effectiveness estimates were reported from other countries by using surveillance data. In the Netherlands, data from 1997 suggested an effectiveness of 51% of the local vaccine against bacteriological proven pertussis (de Melker et al., 2000). In a case-control study in Canada in children aged 4 years and more, 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 a 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 are the reproducible production processes 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 as 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, Kimura et al. 1991, Mortimer et al. 1990, Tomoda et al. 1991, Stehr et al., 1999). The second type of aP vaccines purified the antigens separately and contained equal amounts of FHA and and PT (usually 12.5 to 24 μg per dose). They were initially licensed and used in Japan for children above 2 years of age based on immunogenicity data without an efficacy study.

To evaluate multiple new aP vaccine candidates, a multicenter aP trial was conducted that assessed the safety and immunogenicity of 13 candidate aP vaccines, and two wP vaccines (Decker and Edwards, 1995). Taking into account the results of this immunogenicity study, an array of field studies was performed in subsequent years (Table 2) (cf: Edwards and 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 studies into a synopsis (Edwards 2008). Unfortunately, all trials failed to identify reliable serologic correlates for clinical protection of the individual (see also below).

As a result from 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, which started 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 until the surveillance ended in 2008. (http://www.smittskyddsinstitutet.se/upload/Publikationer/SMI-rapport-2006-4.pdf)
As 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 VEARS in the US 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 the overall health, but troubled parents. A systematic review (Rennels, 2004) showed that this type of side-effect was not typical for aP vaccines, but that all pediatric vaccines 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 vaccines that had experienced limb swelling after the fourth dose, and only 20% had another limb swelling (Rennels et al., 2008). Some cohorts of aP vaccine study participants have now received up to six doses of aP vaccines, 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 also 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 concerning their immunogenicity and their side effects. One of the reduced-dose aP vaccine was tested for its efficacy in a trial among US adolescents and adults and found to have a point estimate of efficacy of 92% (95% CI: 32%-99%) (Le et al., 2005). In another study in UK adolescents, both reduced-dose vaccines in combination with tetanus and diphtheria toxoid (Tdap or Tdap-IPV, respectively) were both immunogenic and safe (Southern et al., 2005). The effectiveness of giving combined TdP-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 has slightly 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 long time been combined with tetanus and diphtheria toxoid into the DTwP vaccine. In few countries, monovalent wP vaccines are still available. Most aP vaccines are combined with other antigens into combination vaccines, and no monovalent aP vaccine is available in the Americas and the EU. 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 i.e. it has been shown that the geometric mean titers of antibodies against *H. influenzae* type b polysaccharide is significantly lower in vaccinees who received combination vaccines. Secondly, combination vaccines have produced regulatory concerns, because their safety and effectiveness may be more difficult to monitor and regulate than single component vaccines. (Decker, Edwards and Bogaerts, 2008). Immunologic theory suggests that the simultaneous exposure of the immune system with multiple conjugate antigens (such as Hib, Streptococcus pneumoniae, and Neisseria meningitis), could result either in either enhanced or suppressed immune responses. Suppression is assumed to occur, when a specific carrier for a polysaccharide is given more than once, and this phenomenon is called carrier-induced epitopic suppression.
Combination vaccines are licensed under the assumption that combining their antigens does not interfere with their safety, immunogenicity and effectiveness. This is mostly not done by efficacy studies but by non-inferiority studies comparing the immunogenicity of separately administered licensed vaccines. A recent example may illustrate possible problems: DTaP-Hib-combination vaccines were introduced in the UK, when another combination with wP was not available. The UK used a 2, 3, 4 months 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; Kalies et al., 2004). This and other experiences suggest that sufficient postmarketing surveillance is needed if vaccination plans are changed from non-combined to combination vaccines.

Safety of combination vaccines so far has be 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 US infants (Kalies et al., 2006, Marshall et al.).

Overall, however, 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 DwPT vaccine. A dose of DwPT 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 IPV vaccines and, experimentally with *Neisseria meningitis* type C vaccine. Many of these combination products are used for primary immunization of infants. (Decker, Edwards and Bogaerts, 2008). Most immune responses to the different antigens were similar, when antigens were either injected separately or as a combination. Antibodies to the 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 and Bogaerts, 2008.
Apart from combination vaccines for primary immunization, reduced dosed combination vaccines have been developed for booster immunization. These vaccines contain about 1/3 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 adults and adolescents was done with a non-combined vaccine (Le et al.,2005); all immunogenicity data and a recent effectiveness study of reduced dose combination vaccines with pertussis components (Rank et al., 2008) suggest that they are as effective as a non-combined product.

**Measuring the Immune Response to B. pertussis Antigens**

Even though wP vaccines have been used successfully for decades, there are no reliable measures of immunity to pertussis. Although many of serological techniques have proven 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 in order to make results comparable. These standardization would include methodology, antigens and reference materials, which has so far only partly been achieved for 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 NIBSC (Xing et al., 2009). All methods used for measuring the immune response to *B. pertussis* antigens are also been 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 of Miller et al. (1943) and Sako (1947) suggested some correlation of agglutinins with immunity; vaccinated children with high agglutinin titers 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 titers 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 titer is above 1:320 than at lower BA titers.

**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 IgG and IgA responses following disease or vaccination (Ashwort et al. 1983, Baraff et al. 1984, Burstyn et al. 1983,
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; Matteo and 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; de Melker et al. 2000; Wirsing von König et al. 1999 and 2002; Prince et al., 2006, Baughman et al., 2005).

All studies that gave recommendations about the use of serology in pertussis diagnosis were performed in populations vaccinated with DTwP vaccines. 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. NEJM 1996; Olin et al. Lancet 1997). Antibody responses to specific antigens are often different in DTaP vaccines compared with DTwP vaccines and therefore last longer (Greco et al. NEJM 1996; Olin et al. Lancet 1997; Guiso et al. Vaccine 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 (Redd et al., 1988; Guiso et al, 1993), but have limitations. Immunoblots can not 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 multicolored beads have been applied to pertussis serology, and offer 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 microtiter cultures of Chinese hamster ovary (CHO) cells. PT induces a distinct cytopathogenic effect resulting in the clustering of CHO cells in the micro plate 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 (Gillenius et al. 1985, Granstrom et al. 1985). The 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 hemagglutination, bactericidal reaction, immunodiffusion, and complement fixation have also been used but overall have not gained wide acceptance (for a review see Onorato and 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 (ICS: intracellular cytokine secretion), or by counting the cytokine producing cells (ELISPOT). These assays have been used also 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 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 how 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 re-infection (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. 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 convincingly shown 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 programs (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, Granstrom et al. 1988, Nagel & Poot-Scholtens 1983, Trollfors et al., 1999; Ward et al., 2005, Watanabe et al, 2006). There is a significant rise of IgG- and IgA-antibodies to PT, FHA and other antigens (Granstrom et al. 1988, Nagel & Poot-Scholtens 1983). In infants, 6 to 7 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.,1988; 1989; Thomas et al., 1989).

### Immune responses after non-primary stimulation

All populations are more or less continuously exposed to *B. pertussis* antigens, and 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, which makes diagnosis more difficult as titer increases may not be seen between acute and convalescent serum samples. As a consequence, titer 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 cutoffs 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 DøPT immunization, which represent transplacentally acquired maternal IgG (Celko et al., 1988; Baraff et al. 1984, Granstrom et al. 1982, 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, Heininger et al., 2009; Healy et al., 2004). 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., 1995). 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 6 doses of unadsorbed wP vaccine, with a total dose of 150 million pertussis
organisms. Most of the newborns showed agglutinin and mouse protective antibody titers equal to or greater than their mothers (Cohen & Scadron 1943). In most of the early studies with unabsorbed vaccine, the total dosage in terms of volume and numbers of organisms was large by today's standard. 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 \textit{H. influenzae} type b, \textit{Streptococcus pneumoniae}, and \textit{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 \textit{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 et al., 2007)

\textbf{Duration of protection after natural infection}

Few studies have attempted to determine the duration of protection after \textit{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 thus difficult to distinguish between the duration of immunity induced by primary infection, and the immunity induced by symptomatic or asymptomatic re-infections. While Gordon and Hood (1951) assumed a near lifelong protection, a cohort study in Germany (WvK et al., 1995) assumed a protection of 15 years, modeling studies assumed a duration of 7-10 years (Miller and Gay, 1997), and a case series from the Netherlands assumed a protection between 3 and 12 years (Versteegh et al., 2002). Case reports of symptomatic reinfections as early as 3.5 years after a previous infection have been published (Versteegh et al., 2002).

\textbf{Antibody decay after natural infection}

Studies from the Netherlands (de Melker et al. 2000; Versteegh et al., 2005), Germany (Heininger et al., 2004), the US (Hodder et al., 2000), and Japan (Tomoda et al., 1992) measuring IgG-anti-PT after infection with \textit{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. Then antibody levels decreased rapidly, so that after 5 years, all subjects had levels of IgG-anti-PT <10 IU/ml. A mathematical model used for the Dutch data predicted that depending of the age of the patient, most of the patients would be below the usual cutoff level of 100 IU/ml after one year (Table 7).

\textbf{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 \textit{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 the US (Marchant et al., 1994; Yih et al., 2000; Baughman et al. 2004), in the Netherlands (de Melker et al., 2000) and Germany (WvK et al., 1999) 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 titers increased in older teenagers, so that 90% of young adults had measurable antibody titers (Granstrom et al. 1982). This is in agreement with the results of studies in the prevaccination era, which showed that a high proportion of children had experienced pertussis infection by age 10 years (Fine & Clarkson 1984). In Palermo, Italy, where the coverage rate with DwPT vaccine was very low, the results of a seroepidemiological study suggest a high exposure of children to *B. pertussis*, resulting in increasing rates of seroprevalence of IgG anti-PT antibodies with age. The overall prevalence of these antibodies determined by the ELISA test was 56%; it increased from 24% in 1 to 3 year old children to 67% in 11 to 12 year old children (Stroffolini et al. 1989).

Astonishingly, in vaccinating countries, such as the US, results of seroepidemiological studies showed similar results in older children, adolescents and adults (Cattaneo et al., 1996). Another study in the US used serum samples from a national nutrition survey and found that antibodies to PT indicated that *B. pertussis* was widely circulating in the population (Baughman et al., 2004). The US 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 persons with ELISA IgG antibody against pertussis toxin also increased with age, from 16% in 5 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 findings concerning the circulation of *B. pertussis* were derived from serosurveys (Stroffolini et al., 1991; 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 titers 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 (Ashworth et al. 1983, Baraff et al. 1984, Barkin et al. 1984, Wilkins and Wehrle, 1987; Blumberg et al. 1991, Grimprel et al., 1996).

The extent of the response was proportionate to the number of doses administered; Elevated level of antibodies to outer membrane protein (OMP) and lipooligosacharide (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 (Provinciano et al., 1965).

In most studies, more than 70% of children responded to three doses of DwPT vaccine with an agglutinin titer of 1:80 or more. However, wP pertussis vaccines from various manufacturers differ considerably in their immunogenicity. The mean agglutinin titer after three doses of DwPT vaccine ranged between 1:1826 (Barkin et al. 1984) and 1: 87 (Blumberg et al. 1991). In a study in France, three doses of DwPT-polio vaccine (adsorbed on calcium phosphate) failed to stimulate an agglutinin level of 1:10 in 25% of children and the mean titer (1:23) was low (Relyveld et al. 1991). A clinical trial conducted at two different academic centers in the US 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 titers (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.


Serological studies provide strong evidence for the booster effect of the fourth dose of DwPT vaccine administered at the end of the second year of life, since antibodies against PT, FHA, and agglutinins increase significantly after the booster dose (Barkin et al. 1984, Chen et al. 1957, Edwards et al. 1989, Pichichero et al. 1987, 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 be always 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 and Decker, 2008). Given the array of clinical presentations of pertussis, assessing the effectiveness of wP vaccines is difficult, and the pitfalls of using immunization registry data to determine vaccine effectiveness has recently been highlighted (Mahon et al., 2008).

In many countries, such as 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 US, the effectiveness of wP vaccine was estimated to be 0.76 (Kenyon et al., 1996). In Poland, it was observed that the effectiveness decreased between 1996 and 2001 for reasons unknown 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 recommended the use of aP vaccines instead (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). In contrast, a study in rural Senegal reported the wP vaccines were more effective (0.67) than a two-component aP vaccine (0.32) (Preziosi and 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 on 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, a number of immunogenicity studies, but no efficacy studies, were performed in adults and in pregnant women (cf Keitel 1999).

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 mostly more pronounced than the response to wP vaccine (Anderson et al. 1988, Edwards et al. 1989, Miller et al. 1990, Morgan et al. 1990, Pichichero et al. 1987, 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 (cf. Edwards and Decker, 2008). Differences have 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 titer, while the response to wP vaccine was strongly dependent on the preimmunization titer. It is not known whether the better response to aP vaccine among those with higher anti-PT titers 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 (Anderson et al. 1988, Edwards et al. 1989, Pichichero et al. 1987) 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 and Decker, 2008).

**Effectiveness of acellular vaccines in infants**

A number of large vaccine efficacy studies with aP vaccines have been performed. 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 thus the results of the studies have repetitively been compared and summarized (cf. Edwards and Decker, 2008). An example of the various estimates of efficacy is given in table 8. Meanwhile with the broad use of aP vaccines in the population, effectiveness estimates are available (Elliott et al., 2004; Edwards and 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 (Hallander et al., 2007, He et al., 2003), 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 (Gustafsson et al., 2006). 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 US (Bisgard et al., 2005) found that an 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 (WvK et al., 2005; 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 *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 aP vaccines for maintaining immunity against pertussis in older children or adolescents has intensively been discussed and many countries have now recommended booster doses in adolescents and adults. A vaccine efficacy study conducted in the US 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 (what country?) had a point estimate of 0.78 (Rank et al., 2009). Given the intensive circulation of the bacteria, even one dose of an 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 EU, as well as Australia, Canada and the US recommend a pre-school and/or an adolescent booster. The US and many other countries have also introduced a booster for the adult population with a Tdap combination vaccine (CDC, 2006; CDC, 2009)

**Correlates of protection for pertussis vaccines**

No serological correlate for protection after vaccination with wP vaccines has been established, although already the MRC trial 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 aP vaccines (Table 9). Derived from data from the Swedish NIH sponsored trial (Storsaeter et al., 1998) it could be shown 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 they found that subjects with anti-PRN titers 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 re-infection (Storsaeter et al., 2003). The Gothenborg 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 strongly advisable not to interchange wP or aP vaccines from different manufacturers during the primary series.

In one study 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 (WvK 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 (Griffith 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 (Silfverman et al., 2007). This is reflected in differences in the immunization schedules used. Usually, the primary series consists of three doses of DwPT vaccine given during the first year of life. In the African and South East Asia Regions, most countries use the immunization schedule recommended by EPI, which calls for three doses of DwPT vaccine at 6, 10 and 14 weeks; some countries use a 3, 4 and 5 months schedule. In the American Region, the schedule used in the United States, with two month intervals between doses in the primary series, is mostly used.

It should be mentioned that for 14% to 18% of countries in the American, Eastern Mediterranean, European, and Western Pacific Regions, the third dose of DwPT vaccine is recommended at a later age, generally after six months of age, so this may reflect a two-ase 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 the time after immunization. In the United Kingdom, the vaccine efficacy fell from 100% in the first year following three doses of DwPT vaccine to 46% in the seventh year (Jenkinson 1988). In another outbreak study in the US it was estimated that protection lasts about 12 years after wP vaccination. In Sweden, the efficacy of three doses of unadsorbed wP pertussis vaccine declined from 89% in 6 to 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 5 and 14 years, and these studies are summarized in table 10.
About one-third of countries in the American and Western Pacific Regions give a fifth dose of DwPT vaccine ("second booster"). The need for a fifth dose of DwPT vaccine and its importance in controlling pertussis in other regions remains to be proven. Serologically, this additional dose of DwPT vaccine seems to exert a clear booster effect (Edwards et al. 1989, Morgan et al. 1990).

Antibody decay after immunization with whole cell vaccines

Few studies have addressed the antibody decay after vaccination with wP vaccines. Most data are available for aP vaccines (Edwards et al., 1995; Edwards et al., 1991). Overall, the relatively low levels of antibodies induced by wP vaccines rapidly decline below detection levels one to two years after vaccination (Blennow and 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 6 years of age. Gustafsson et al. (2006) 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 (Sweden, Germany, Senegal) (Table 10). Thus, it has been suggested that a school-entry vaccination is necessary after a three-dose primary course of immunization (Carlsson and Trollfors, 2009). Some countries, i.e. Australia have even skipped the first booster in the second year of life in favor of a pre-school 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 Sweden and Italy also produced data about the antibody decay in the study populations. Giulinao 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: dTap boosters result in rapid responses to pertussis antigens in adults (Kirkland et al., 2009). The APERT study (Le et al., 2005) suggested that these antibodies to PT will be above the level of detection for about 5 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 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 GMC of 83 EU/ml IgG-anti-PT that decreased to 30 EU/ml after one year. Edelman et al. (2004, 2007) using the same vaccine in adolescents found a peak GMC of IgG-anti-PT of 116 EU/ml four weeks after vaccination, which decreased to 16 EU/ml three years and to 8 EU/ml five years after vaccination. Riffelmann et al. (2009) vaccinated HCW and found a higher peak value, with a rapid decline in antibody over the first year after vaccination with 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**

As wanted?

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AC</td>
<td>adenylate cyclase</td>
</tr>
<tr>
<td>AGG</td>
<td>agglutinogens</td>
</tr>
<tr>
<td>aP</td>
<td>acellular pertussis vaccine</td>
</tr>
<tr>
<td>DTP</td>
<td>diphtheria-tetanus-pertussis vaccine</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FHA</td>
<td>filamentous hemagglutinin</td>
</tr>
<tr>
<td>HCW</td>
<td>Health care worker</td>
</tr>
<tr>
<td>HLT</td>
<td>heat-labile toxin</td>
</tr>
<tr>
<td>Lf</td>
<td>floculation units of toxoid</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>PRN</td>
<td>pertactin</td>
</tr>
<tr>
<td>PT</td>
<td>pertussis toxin</td>
</tr>
<tr>
<td>Tdap</td>
<td>DTaP with reduced antigen content</td>
</tr>
<tr>
<td>wP</td>
<td>whole cell pertussis vaccine</td>
</tr>
</tbody>
</table>
Figure 1:

Diagrammatic sketch of some *B. pertussis* virulence factors

(Courtesy Dr. Heinz-Josef Schmitt)
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<tr>
<th>Virulence factor</th>
<th>Function / structure</th>
<th>vaccine component</th>
<th>antibody isotypes</th>
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<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>
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<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>
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<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>
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<tr>
<td></td>
<td></td>
<td>Surface protein</td>
<td></td>
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<td>PT</td>
<td>suppression of host response</td>
<td>wP, all aP</td>
<td>IgG, IgA</td>
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<tr>
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<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>
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<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>
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<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>
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Table 2: Composition of some acellular vaccines and efficacy estimates (Edwards and Decker, 2008)

<table>
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<tr>
<th>Acellular vaccine</th>
<th>Composition</th>
<th>Producer</th>
<th>Study site</th>
<th>% VE</th>
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<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-Imune</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>
<tr>
<td>Method</td>
<td>quantification</td>
<td>antigens</td>
<td>isotypes</td>
<td>reported unit</td>
</tr>
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<td>---------------------</td>
<td>----------------</td>
<td>-------------------------------</td>
<td>-------------------</td>
<td>---------------</td>
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<tr>
<td>ELISA</td>
<td>yes</td>
<td>PT, FHA, PRN, FIM</td>
<td>IgG, IgA</td>
<td>IU/ml</td>
</tr>
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<td>Immunoblot</td>
<td>semiquantitative</td>
<td>PT, FHA, ACT</td>
<td>IgG, IgA</td>
<td>qualitat.</td>
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<tr>
<td>Agglutination</td>
<td>semiquantitative</td>
<td>whole cells</td>
<td>IgM (IgG)</td>
<td>titres</td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>yes</td>
<td>PT, FHA, PRN</td>
<td>IgG, IgA</td>
<td>IU/ml</td>
</tr>
<tr>
<td>CHO-cell assay</td>
<td>semiquantitative</td>
<td>PT</td>
<td>IgG</td>
<td>Titres</td>
</tr>
<tr>
<td>IFT</td>
<td>semiquantitative</td>
<td>whole cells</td>
<td>?</td>
<td>Titres</td>
</tr>
<tr>
<td>Clinical symptoms</td>
<td>Duration</td>
<td>confirmation</td>
<td>Intended use</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td>----------</td>
<td>-------------------------</td>
<td>----------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Paroxysmal cough</td>
<td>&lt;21 days</td>
<td>positive culture</td>
<td>Efficacy trials</td>
<td>WHO, 1991</td>
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<tr>
<td></td>
<td></td>
<td>Increase of IgG/IgA anti-PT / FHA / AGG2/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contact with culture proven case</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></td>
<td></td>
<td>positive PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contact with proven case</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ posttussive vomiting</td>
<td></td>
<td></td>
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<tr>
<td>Pertussis diagnosed by a physician</td>
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<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>
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<td></td>
<td>positive paired serology</td>
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<td></td>
<td></td>
<td>+ posttussive vomiting</td>
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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>cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>MA, USA</td>
<td>population study</td>
<td>~ 100 IU/ml</td>
<td>##%</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>##%</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>WvK 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>
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</tr>
<tr>
<td>AUS</td>
<td>clinical validation</td>
<td>50 IU/ml</td>
<td>better than 100 IU/ml</td>
<td>Horby et al., 2005</td>
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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 UK</td>
<td>Gordon and Hood 1951</td>
</tr>
<tr>
<td>1995</td>
<td>Household contact</td>
<td>15-20</td>
<td>Germany</td>
<td>Wirsing von König et al., 1995</td>
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<tr>
<td>2002</td>
<td>Case series</td>
<td>3.5 - 12</td>
<td>Netherlands</td>
<td>Versteegh et al., 2002</td>
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</table>
Table 7: Antibody decay after natural infection as compared to 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>
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<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>
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<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>
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Table 8: Estimated degree of protection achieved after different doses of pertussis vaccines

<table>
<thead>
<tr>
<th>Year of study</th>
<th>Type of study</th>
<th>Vaccine</th>
<th>Dose</th>
<th>Endpoint</th>
<th>VE</th>
<th>Country</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1990</td>
<td>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></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.80</td>
</tr>
<tr>
<td>2004</td>
<td>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></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.801</td>
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<td></td>
<td>0.933</td>
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<td>1996-98</td>
<td>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>
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<tr>
<td></td>
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<td></td>
<td>0.918</td>
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<td></td>
<td></td>
<td>0.998</td>
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<td>1990-2004</td>
<td>Surveillance</td>
<td>wP</td>
<td>1</td>
<td>hospitalization</td>
<td>0.36</td>
<td>Denmark</td>
<td>Hviid, 2009</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>0.66</td>
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<td></td>
<td></td>
<td></td>
<td>0.87</td>
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<tr>
<td>1990-2004</td>
<td>Surveillance</td>
<td>ap</td>
<td>1</td>
<td></td>
<td>0.51</td>
<td></td>
<td></td>
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<td>1990-2004</td>
<td>Surveillance</td>
<td>ap</td>
<td>2</td>
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</tr>
<tr>
<td>1990-2004</td>
<td>Surveillance</td>
<td>ap</td>
<td>3</td>
<td></td>
<td>0.96</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>
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<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) household contact</td>
<td>wp (?)</td>
<td>high titres protect</td>
<td>Deen et al. 1995</td>
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</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., 1994</td>
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Table 10: Estimated duration of protection after vaccination (three doses)

<table>
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<tr>
<th>Year</th>
<th>vaccine</th>
<th>type of study</th>
<th>duration</th>
<th>country</th>
<th>reference</th>
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<tr>
<td>1993</td>
<td>wP</td>
<td>surveillance</td>
<td>8 years</td>
<td>UK</td>
<td>Jenkinson et al., 1988</td>
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<tr>
<td>1996</td>
<td>wP</td>
<td>surveillance</td>
<td>5-10</td>
<td>SF/CH</td>
<td>He et al., 1996</td>
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<tr>
<td>1999</td>
<td>wP</td>
<td>surveillance</td>
<td>5-14</td>
<td>UK</td>
<td>van Buynder et al., 1999</td>
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<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>
<tr>
<td>Type of aP vaccine</td>
<td>% left</td>
<td>12 mo</td>
<td>18 mo</td>
<td>36 mo</td>
<td>48 mo</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td><strong>Tdap</strong>: 2.5µg PT, 5µg FHA, 3µg PRN, 5µg FIM, d, T</td>
<td></td>
<td>35%</td>
<td>30%</td>
<td>18%</td>
<td></td>
</tr>
<tr>
<td><strong>Tdap</strong>: 8µg PT, 8µg FHA, 2.5µg PRN, d, T</td>
<td></td>
<td>36%</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>Tdap</strong>: 8µg PT, 8µg FHA, 2.5µg PRN, d, T</td>
<td></td>
<td>n.d.</td>
<td>n.d.</td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td><strong>Tdap</strong>: 8µg PT, 8µg FHA, 2.5µg PRN, d, T</td>
<td></td>
<td>27%</td>
<td>27%</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>aP</strong>: 25µg PT, 25µg FHA, 7µg PRN</td>
<td></td>
<td>29%</td>
<td>n.d.</td>
<td>19%</td>
<td>20%</td>
</tr>
</tbody>
</table>