

# **The immunological basis for immunization series**

## **Module 3: Tetanus Update 2006**

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**Immunization, Vaccines and Biologicals**



**World Health  
Organization**

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# Abbreviations and acronyms

ACIP	Advisory Committee on Immunization Practices
AIDS	acquired immunodeficiency syndrome
CDC	Centers for Disease Control and Prevention (USA)
Da	Dalton (mass spectrometry)
DTP	diphtheria-tetanus-pertussis vaccine
DT	diphtheria-tetanus vaccine for children
EPI	Expanded Programme on Immunization
ELISA	enzyme-linked immunosorbent assay
GBS	Guillain-Barre Syndrome
HA	passive haemagglutination test
Hib	<i>Haemophilus influenzae</i> type b
HIV	human immunodeficiency virus
Ig	immunoglobulin
IOM	Institute of Medicine (USA)
IU	international units
LF	limits of flocculation
mL	millilitre
MNT	maternal and neonatal tetanus
NA	neutralization assay
ng	nanogram
PRP	polyribosylribitol phosphate
RIA	radioimmunoassay
RSV	respiratory syncytial virus

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Td	preparation of diphtheria and tetanus toxoid with a low amount of diphtheria toxoid, for adolescents and adults
TdaP	preparation of diphtheria, tetanus toxoid and acellular pertussis with a low amount of diphtheria toxoid, for adolescents and adults
ToBI	toxin binding inhibition test
TT	tetanus toxoid
TT2+	second dose of tetanus toxoid
UNICEF	United Nations Children's Fund

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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)<sup>1</sup>. 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, i.e. diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. These 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 (2005-2015) ([http://www.who.int/vaccines-documents/DocsPDF05/GIVS\\_Final\\_EN.pdf](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 has been 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 WHO recommendations on vaccine use that since 1998 are published in the Vaccine Position Papers ([http://www.who.int/immunization/documents/positionpapers\\_intro/en/index.html](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.

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<sup>1</sup> This programme was established in 1974 with the main aim of providing immunization for children in developing countries.

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# 1. Tetanus

## 1.1 Tetanus toxin

Tetanus is caused by the action of a highly potent neurotoxin, tetanospasmin, which is produced during the growth of the anaerobic bacterium *Clostridium tetani*. *Cl. tetani* is not an invasive organism; infection with *Cl. tetani* remains localized. The disease usually occurs through infection of a skin injury with tetanus spores. Tetanus spores introduced into an area of injury germinate to tetanus bacilli in the presence of necrotic tissue with reduced oxygen potential. Neonatal tetanus occurs through infection of the umbilicus when the cord is cut with an unclean instrument or when substances heavily contaminated with tetanus spores are applied to the umbilical stump.

Tetanus toxin is produced by *Cl. tetani* bacteria as a single polypeptide chain of 150 000 Da molecular weight when cleaved to two linked polypeptides and is neurotropic, binding specifically to ganglioside-containing receptors at nerve termini. It is extremely potent; the estimated human lethal dose is less than 2.5 ng per kg. The toxin migrates to its site of action in the central nervous system by retrograde axonal transport within nerve cells. Once inside neurons, tetanus toxin cannot be neutralized by tetanus antitoxin. Toxin accumulates in the central nervous system, where it prevents the release of inhibitory neurotransmitters, such as glycine and gamma-aminobutyric acid, thereby leaving excitatory nerve impulses unopposed.

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## 2. Tetanus toxoid and the nature of immunity against tetanus

### 2.1 Tetanus toxoid vaccine

Tetanus toxin can be inactivated by formaldehyde to yield tetanus toxoid. Tetanus toxoid has been used as a monovalent vaccine (TT) to immunize adults, or as a component of combined diphtheria-tetanus-pertussis (DTP) vaccine or diphtheria-tetanus (DT) vaccine for immunization of children. A combined tetanus-diphtheria (Td) vaccine for adults contains the equivalent amount of tetanus toxoid and a reduced amount of diphtheria toxoid compared to DTP or DT vaccines, and is recommended for use instead of monovalent tetanus toxoid in order to increase population immunity to diphtheria. Tetanus toxoid may also be administered as a component of a tetanus-diphtheria-acellular pertussis (Tdap) combination, primarily focused at better control of pertussis, to adolescents or adults. Tetanus toxoid is adsorbed onto aluminium salts (aluminium hydroxide or aluminum phosphate) to increase its antigenicity. Tetanus toxoid has also been incorporated, along with diphtheria and pertussis, into new combination vaccines, combining up to five of the recommended childhood vaccines. The potent immunogenicity of tetanus toxoid has led to its use as a protein carrier in polysaccharide-protein conjugate vaccines (see Section 9 – Combination vaccines and concomitant vaccine use). Tetanus toxoid is stable, can withstand exposure to room temperature for months, and to 37°C for a few weeks without a significant loss of potency (Dietz et al. 1997; Galazka et al. 1998).

Work is in progress on the development of a slow-release tetanus toxoid that may provide long-lasting immunity with only one injection. This research involves incorporation of tetanus toxoid into injectable and biodegradable microspheres made of well-tolerated polymers. Following injection of the slow-release product, the tetanus toxoid would be released from the injection site at predetermined intervals. An animal model has been developed to assess the immunogenicity of single-dose, controlled-release tetanus and diphtheria vaccines (Gupta et al. 1998). Several experimental vaccines have been investigated (Johansen et al. 2000; Peyre et al. 2003; Jaganathan et al. 2005; Kipper et al. 2006) including one combining routine childhood vaccines with a synthetic malaria peptide antigen (Peyre et al. 2004), but to date there are no data available from clinical trials in humans.

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## 2.2 Tetanus toxoid-induced immunity

Tetanus toxoid induces the formation of specific antitoxins. These antibodies play an important role in protecting against tetanus. Immunity to tetanus is antibody-mediated, with tetanus antitoxins, like diphtheria antitoxins, belonging to the immunoglobulin G (IgG) class; they are distributed throughout the bloodstream and extravascular spaces. Antitoxin in tissues can neutralize toxin produced in an infected wound. Antitoxin which passes to the fetus through the placenta following active immunization of the mother can prevent neonatal tetanus.

Immunity to tetanus toxin is induced only by immunization; recovery from clinical tetanus does not result in protection against further attacks. A small amount of tetanus toxin, although enough to cause the disease, is insufficient to stimulate antibody production. Therefore, all patients with clinical tetanus should be immunized with tetanus toxoid, either at the time of diagnosis or during convalescence. Some authors have proposed that natural immunity could occur following asymptomatic colonization of the intestinal tract (Dastur et al. 1981; Matzkin & Regev, 1985; Tenbroeck & Bauer, 1923; Veronesi et al. 1975, 1981). However, studies have shown that tetanus antibodies in persons who are said to be unvaccinated cannot exclude the possibility of prior, unreported, vaccination (MacLennan et al. 1981); some studies have used *in vitro* techniques and found very low levels of tetanus antibodies that could be due to cross-reaction with other antigens (Dastur et al. 1981; Ray et al. 1978; Matzkin & Regev, 1985). Studies in African schoolchildren (Rey, 1981), Indian military recruits (Menon et al. 1976), persons taking care of horses (Lahiri, 1939), pregnant women in New Guinea (MacLennan et al. 1965), and healthy persons in Upper Volta (Breman et al. 1981), have demonstrated that populations in developing countries with a high level of exposure to tetanus spores usually lack tetanus neutralizing antitoxins. Even if asymptomatic colonization and infection of the intestine with tetanus organisms occurs in some areas of the developing world, natural immunity is not thought to have any practical importance in controlling tetanus.

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# 3. Techniques to measure antibody response

## 3.1 Neutralization test in vivo

The detection of anti-tetanus antibodies by an in vivo neutralization assay is considered to be the “gold standard” methodology due to the fact that it is a measurement of biologically active antitoxin in serum. The neutralization assay is sensitive, detecting as little as 0.001 international units per millilitre (IU/mL) of neutralizing antibody.

The assay is normally performed in mice which are injected with a series of dilutions of test sera incubated with a lethal dose of tetanus toxin. Results in IU/mL are generated by standardization against an international reference sera (Sesardic et al. 1993). Despite the general acceptance of the in vivo neutralization assay as the “gold standard”, variation in the methodology does occur. The subjective nature of the end-points selected for the assay, for example the well-being of mice, can influence outcome and hence antibody titres. Furthermore, the accuracy of the assay is dependent upon the nature of the toxin, the toxin test and weight of mice (Gupta et al. 1985; Peel 1980). It is clear therefore, that although considered to be the “gold standard” assay, there is no internationally standardized protocol available and thus it is very difficult to compare results directly from different studies (and antibody titres) performed by different laboratories. Due to the expensive and labour-intensive nature of the in vivo assay and the need for large numbers of animals, it is unlikely that an internationally standardized protocol will be developed.

## 3.2 In vitro techniques

The interaction between tetanus antibody and tetanus toxin (or toxoid) may be measured in vitro by the passive haemagglutination test (HA), the enzyme-linked immunosorbent assay (ELISA), or the radioimmunoassay (RIA). These techniques are simple, sensitive, rapid, and inexpensive, but they are generally less specific than the in vivo neutralization method. Some in vitro techniques are more sensitive in detecting IgM antibodies than IgG antibodies, particularly in the early period of the primary response; however IgM antitoxin has been shown to be non-neutralizing. Therefore, the results of in vitro techniques should be interpreted carefully and verified against the in vivo neutralization method.

---

### 3.2.1 *Passive haemagglutination*

The HA test is a simple *in vitro* assay where tetanus toxoid-sensitized red blood cells agglutinate in the presence of tetanus antibodies. The reliability of the HA test is limited by the fact that it preferentially measures IgM (Newell et al. 1971; Edsall, 1976) which does not neutralize tetanus toxin (Ourth & MacDonald, 1977). Correlation between the HA test and the neutralization assay has been varied (Levine & Wyman, 1964; Chatterjee, 1964; Hardegree et al. 1970; Winsnes & Christiansen, 1979; Gupta et al. 1984; Gupta et al. 1985). A good correlation occurs with sera containing high or moderate titres but at low titres there may be an overestimation by the HA test due to the detection of non-functional antibody. The HA test is used less now in the determination of antitoxin titres.

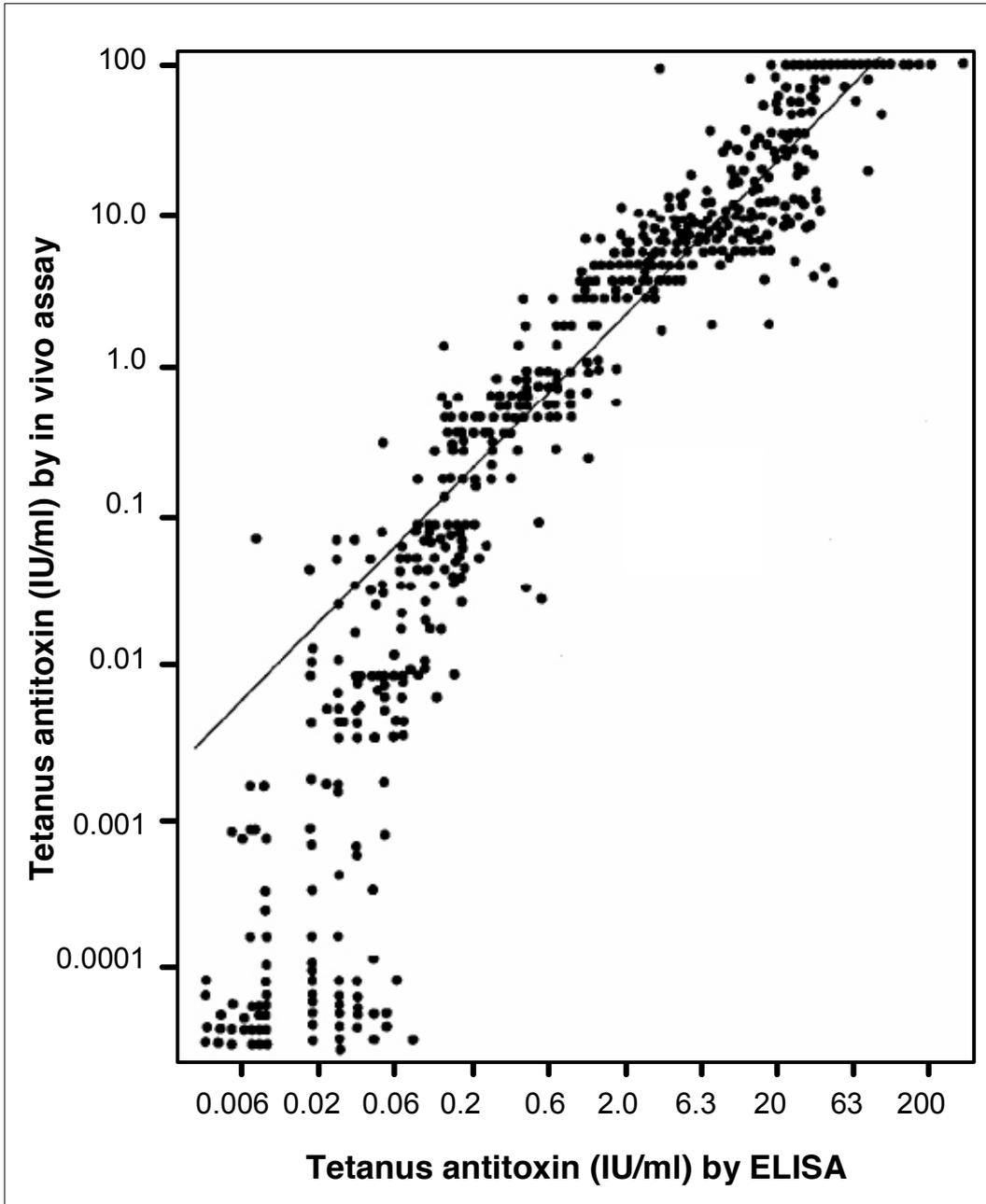
### 3.2.2 *ELISA*

ELISAs are the most commonly used assays to detect anti-tetanus IgG antibodies. An indirect ELISA, where antibody present in the test sera binds to tetanus toxoid bound to a solid-phase (microtitre plate well surface) has been described (Melville-Smith et al. 1983; Sedgwick et al. 1983; Simonsen et al. 1986) and extensively used (see Wassilak et al. 2004). Good correlation between the indirect ELISA and the neutralization assay has been demonstrated (Simonsen et al. 1986; Gupta & Siber, 1994), although this is generally when antibody concentrations are above 0.16–0.2 IU/mL (Simonsen et al. 1986). The indirect ELISA overestimates titres below this range when compared to the neutralization assay (Melville-Smith et al. 1983; Sedgwick et al. 1983; Cox et al. 1983; Hagenaaars et al. 1984; Simonsen et al. 1986; Virella et al. 1991; Dokmetjian et al. 2000). Data from Simonsen et al (1986) implies that the lowest ELISA value reliably predictive of protection was 0.16 IU/mL (Figure 1). This has important consequences in the definition of a protective antibody level (see Section 4).

The overestimation of tetanus antitoxin levels and lack of specificity of the ELISA could be attributed to several factors, such as non-specific binding of antibody to contaminants in the antigen preparation, or recognition of non-biologically important epitopes which may be an artifact created in the antigen preparation (Simonsen et al. 1986; 1987b). Detection of antitoxin of a lower avidity which is insufficient for toxin neutralization *in vivo* may also contribute to the overestimation of titres. A further explanation may be the detection of asymmetric, functionally monovalent, IgG antibodies that have limited toxin neutralizing activity (Dokmetjian et al. 2000).

A competition ELISA was developed with the aim of improving the detection of biologically relevant antibodies and improving the correlation with the neutralization assay (Simonsen et al. 1987b). The competition ELISA involves mixing test sera with tetanus toxoid and allowing the mixture to react with bound toxoid on ELISA plates. The quantity of antibody capable of binding to both free and bound toxoid is then determined and compared to that of a standard. The modified assay improved the correlation to the neutralizing assay (correlation coefficient of 0.98) but it was unclear if this was because the format enabled the detection of antibodies with a higher avidity and in theory functional, or whether it corrects for the presence of non-specific antibodies (Simonsen et al. 1987b).

**Figure 1. Tetanus antitoxin levels measured in 727 sera by ELISA and by an in vivo assay**



Source: Galazka, 1993 (Original data from Simonsen et al.1986).

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A toxin binding inhibition (ToBI) assay has been reported and demonstrated to show good correlation with the neutralization assay (correlation coefficient = 0.95) (Hendriksen et al. 1988). The assay determines the level of inhibition of binding of tetanus toxoid to a polyclonal antitoxin by tetanus antibodies in the test sera.

More recently, a double antigen assay format has been developed which does not require any additional specialized equipment compared to the traditional ELISA (Aggerbeck et al. 1996; Kristiansen et al. 1997). Anti-tetanus antibodies in test sera are detected if bound to the solid phase tetanus toxoid and a labeled tetanus toxoid in solution. It is hypothesized that the assay correlates better with neutralization assays due to the requirement that antibody must bind to two separate toxoid molecules to be detected, which may mimic the requirements for in vivo neutralization of toxin. The double antigen assay has been used in a serological survey of tetanus antibodies in individuals of various ages in Australia and the Republic of Turkey (Gidding et al. 2005; Caglar et al. 2005) and assessment of the serological protection of mothers of young children in the Central African Republic, demonstrating its potential for analysis of a large number of samples (Deming et al. 2002).

### *3.2.3 Other tests*

Other assays have been developed including radioimmunoassay (Stiffler-Rosenberg & Fey, 1975) and, more recently, flow cytometric assays utilizing fluorescent microspheres (Pickering et al. 2002), but the most commonly used remains the indirect ELISA, either in-house or in kit format. This has obvious implications in the interpretation of results due to the limitations of this assay format (see Section 4).

## **3.3 Standardization**

Standardization of assays between laboratories and the production of an internationally recognized methodology would provide a basis for comparison of data between studies. Hendriksen & Winsnes (2002) reported on an interlaboratory comparison of ELISA and ToBI assays which demonstrated that differences were generally less than two-fold. However, interpretation of historical data remains critical and requires caution because the type of assay used to generate the data should always be taken into consideration.

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## 4. “Protective Level” of tetanus antibodies

For most infections, laboratory markers of immunity which reliably predict protection from clinical disease in field studies are used as predictors of vaccine efficacy. For clarity, the marker has to consistently predict protection at an individual level and actually mediate the protection observed. It has been suggested that a surrogate is the measurement of a functionally protective laboratory marker, and a correlate is the measurement of a marker, usually by a non-functional assay which correlates strongly with the surrogate of protection (Borrow & Miller, 2006). Hence, the measurement of toxin-neutralizing antibody would be regarded as a surrogate of protection and detection of antitoxin (toxoid) specific IgG would be considered a correlate to the surrogate of protection. Surrogates of protection can be obtained from studies of natural immunity, Phase III efficacy trials, or passive immunization. For tetanus, the existence of natural immunity is questionable, and large-scale efficacy studies have rarely been performed with concomitant measurement of antibody. These data on protective levels have therefore been subject to much debate.

It has often been accepted that the minimum level of antibody required for protection is 0.01 IU/mL measured by an *in vivo* neutralization assay. Where did this level actually come from? As mentioned, Sneath et al (1937) are credited first with hypothesizing that this level would be sufficient to prevent disease in man. They showed that active immunization of guinea pigs induced a level of 0.01 IU/mL which prevented death. They extrapolated from these results to suggest that a similar level would be protective in humans. It is interesting that Sneath et al (1937) noted that 13% of guinea pigs developed clinical tetanus despite antibody levels as high as 0.1 to 0.5 IU/mL. Actual data from human studies are limited. Wolters & Dehmel (1942) immunized themselves, determined their antitoxin levels to be 0.007 to 0.01 U/mL and then challenged themselves with “2–3 fatal” doses of *Cl. tetani* spores without experiencing any clinical symptoms. As it is unclear as to the level of toxin required to cause infection, interpretation of these data should be cautious. Supporting evidence for 0.01 IU/mL as the protective threshold is limited. Looney et al (1956) summarized the attempts made to determine a protective level of antitoxin by reviewing various studies on active immunization experiments in guinea pigs and horses (Ramon, 1936; Sneath et al. 1937; Cowles, 1937; Wolters & Dehmel, 1938; Shumacker & Lamont, 1942; Zuger et al. 1942), and passive immunization data (Sneath & Kerslake, 1935; Gold, 1937; Sachs, 1952), and concluded that “no final answer is at hand”. The experience of the British army during the first World War, where levels of approximately 0.03–0.06 U/mL were achieved by administration of antitoxin and few cases of tetanus occurred in soldiers, has been interpreted as suggesting that those levels were protective (Turner et al. 1954). Tasman & Huygen (1962) suggested again that 0.01 U/mL was appropriate for protection following a review of the literature and applied this criterion to their

study of active immunization of patients treated with anti-tetanus serum. Further support for a protective level is given by the study of MacLennan et al. (1965) who reported that a maternal antitoxin level at delivery of 0.01 U/mL, determined by a neutralization assay, is protective.

The difficulty in assigning a definitive level of antibody for protection is illustrated by the number of cases of tetanus that have occurred in individuals with antibody levels greater than 0.01 IU/mL by neutralization assay, or 0.15 IU/mL by ELISA (Table 1).

**Table 1. Cases of tetanus despite protective levels of antitoxin**

Reference	Year	Observations	Assay
Goulon et al.	1972	9 tetanus patients had levels 0.01–0.1 IU/mL; 1 had level between 0.1–1.0 IU/mL (54 patients had levels <0.01)	NA
Berger et al.	1978	Patient had level of 0.04 IU/mL at onset	ELISA
Passen & Andersen	1986	Patient had level of 0.16 IU/mL at onset	ELISA
Maselle et al.	1991	7 patients had levels of 0.04–0.13 IU/mL	
Crone & Reder	1992	3 patients had levels of 0.15–25 IU/mL – (One had <0.01 IU/mL by NA)	ELISA
de Moraes-Pinto et al.	1995	9 neonates had levels >0.01 IU/mL NA (ELISA ranges: neonates 0.07–2.83; mothers 0.28–4.81)	NA
Pryor et al.	1997	Patient had level of 1.0 IU/mL	NA
Abrahamian et al.	2000	Patient had level of 0.16 IU/mL	ELISA

*NA, neutralization assay*

Other approaches to defining a correlate of protection include taking a population-based approach, in which a comparison is made between antibody levels in a protected group (immunized), versus a susceptible (non- or partially-immunized) group. An antibody level that is exceeded by the majority of the protected individuals and not by the majority of the susceptible population should be validated against the relative risk of disease at the defined titre. This has been illustrated for pertussis and respiratory syncytial virus (RSV) (Siber, 1997), meningococci (Borrow & Miller, 2006) and pneumococci (Jodar et al. 2003a). To date, such studies have not been performed for tetanus, and the relatively rare occurrence of tetanus, combined with the lack of a fully standardized and readily used assay that correlates with toxin neutralization, would make these studies difficult.

In summary, the minimum amount of circulating antitoxin that in most cases ensures immunity to tetanus is assay-specific. With *in vivo* neutralization tests or modified ELISA assays, concentrations exceeding 0.01 IU/mL are usually considered protective, whereas antitoxin concentrations of at least 0.1–0.2 IU/mL are defined as positive when standard ELISA techniques are used for this assessment. Cases of tetanus have been documented, however, in persons with antitoxin concentrations above these thresholds. Hence, a “protective antibody concentration” may not be considered a guarantee of immunity under all circumstances. The aim should be to sustain high antibody concentrations throughout life.

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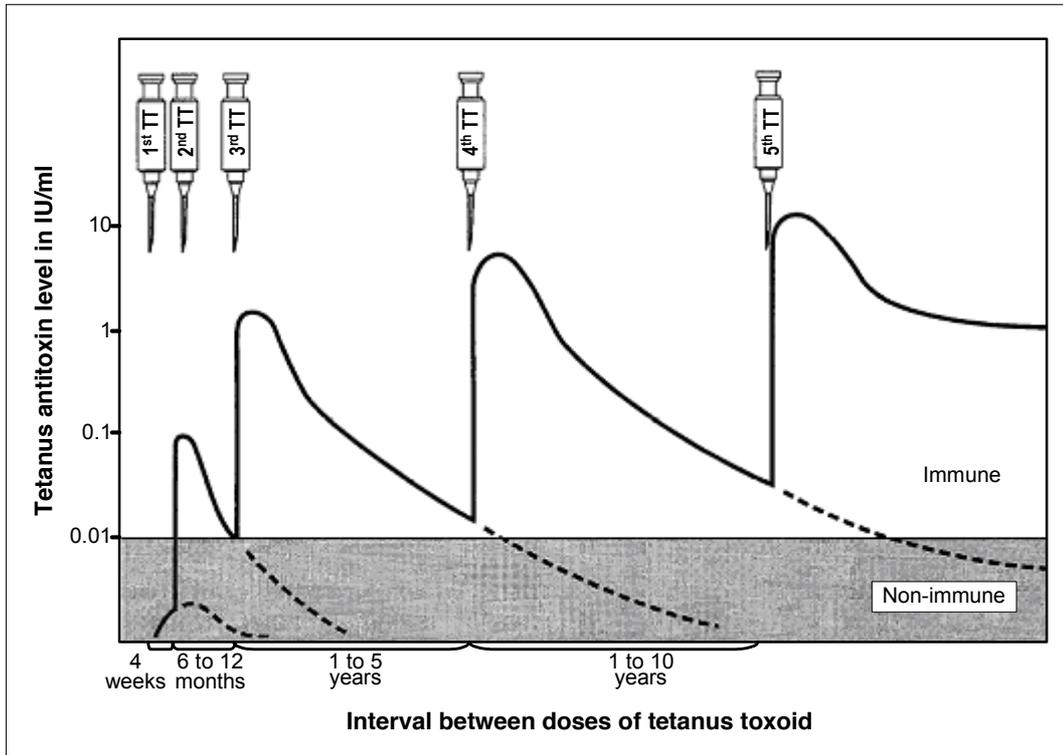
# 5. Development of immunity following immunization

## 5.1 Immune response to immunization

A schematic picture of tetanus antitoxin response of adults following primary and booster immunization with tetanus toxoid is shown in Figure 2. The degree and duration of immunity increases with the number of tetanus toxoid doses given. One dose of tetanus toxoid ensures little, if any, protection. Two to four weeks after the second dose the mean level of tetanus antitoxin usually exceeds the minimum “protective” level of 0.01 IU/ml, although the percentage of poorly-protected persons can still be up to 10%. Immunity also declines with time. After one year the percentage of poorly-protected persons may increase to 20% and the mean titre may fall to the threshold level. A study in Papua New Guinea showed that 78% of women immunized during pregnancy with two 10 limits of flocculation (Lf) doses of adsorbed tetanus toxoid, had antitoxin levels above 0.01 IU/ml for at least three years; the mean antitoxin level was about 0.03 IU/ml (Figure 3). The infants of women with a suboptimal level of antitoxin may be at risk of tetanus. For this reason, a third dose of tetanus toxoid should be given during the subsequent pregnancy, or 6 to 12 months after the initial two doses. A third dose of tetanus toxoid induces plentiful antitoxin production, with mean levels between 1 and 10 IU/ml. The level of immunity induced by a course of three injections is high and durable. One month following the third dose the percentage of poor responders is negligible and the protective level lasts for at least five years. After the third dose, each additional dose given with at least a one-year interval increases the tetanus antitoxin level and prolongs the duration of immunity. Immunity will last for 10 years after the fourth dose and for at least 20 years after the fifth dose.

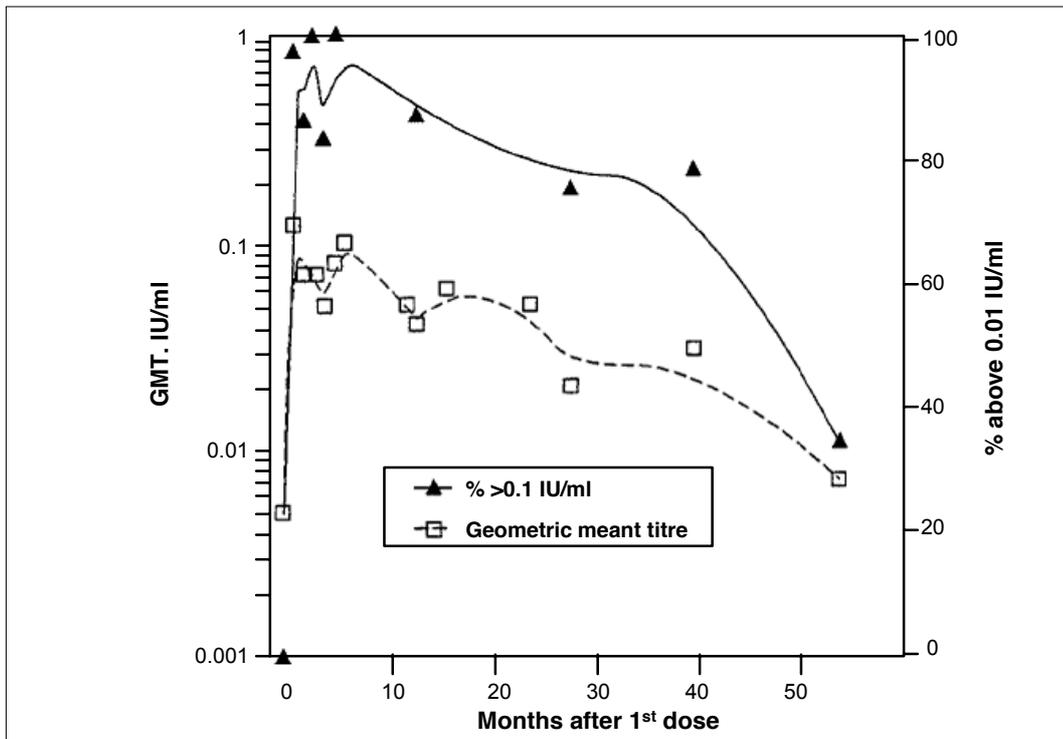
In children, three primary doses of DTP vaccine induce an antibody level above the minimum protective threshold, with a mean level above 0.2 IU/ml (Anderson et al. 1988; Barkin et al. 1984; Edwards et al. 1989; Pichichero et al. 1986). Factors influencing the height of the immune response in children and adults, apart from the number of doses, are discussed in Sections 5.4 and 7.

**Figure 2.** Antibody response to tetanus toxoid (TT)



Source: Galazka, 1993

**Figure 3.** Geometric mean titre and the percentage of pregnant women with 0.01 IU/mL or more of tetanus antitoxin after two doses of adsorbed tetanus toxoid, Papua New Guinea



Sources: Galazka, 1993 (Original data from MacLennan et al. 1965, Hardegee et al. 1970).

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## 5.2 Duration of immunity following various immunization schedules

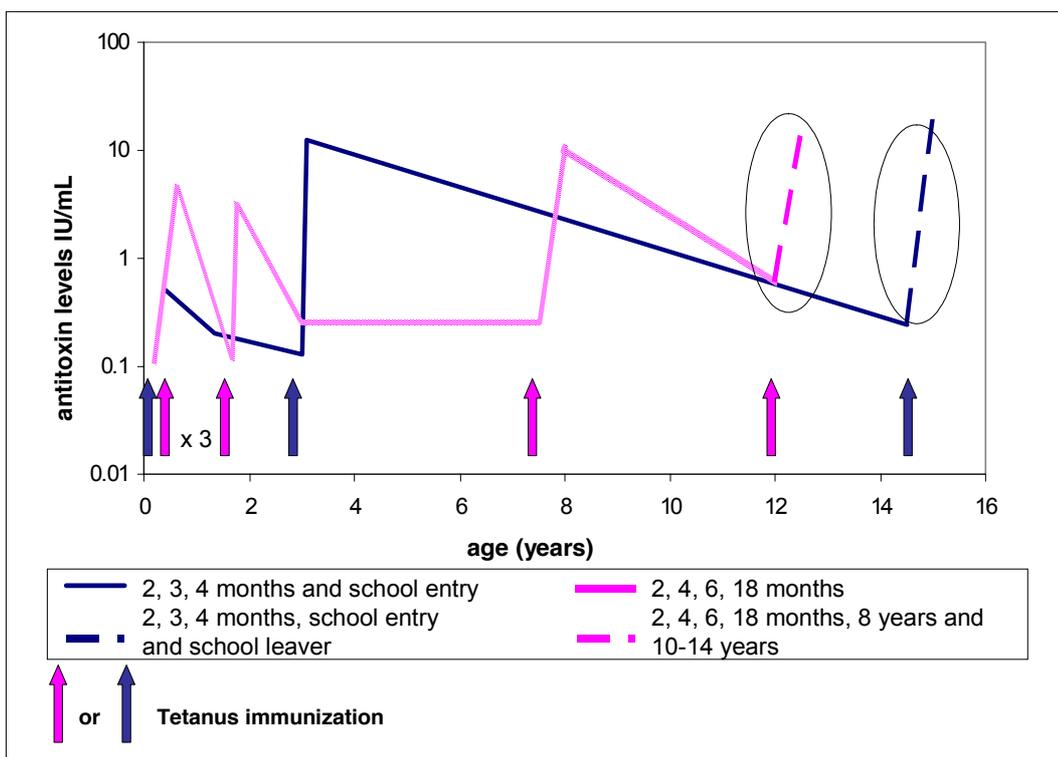
An understanding of the duration of immunity induced by immunization has important implications for the recommendations on the number and timing of doses to be given.

Most data derive from studies on antibody profiles at different time points after vaccination. Data on the duration of clinical protection after vaccination in pregnancy, reported from the People's Republic of Bangladesh (Koenig et al. 1998), suggested that neonatal tetanus mortality rates remained significantly lower in women who had received either one or two injections of tetanus toxoid for up to 12 or 13 years after vaccination. However, these data must be interpreted with caution as the vaccination history of the study subjects is uncertain, and the data contradicts the widely accepted view that multiple doses are required for long-term protection.

Serological data from the United Kingdom (UK) and United States of America (USA) illustrate antibody profiles after two different vaccination approaches (Figure 4). In the UK, three doses are given at 2, 3 and 4 months of age, and then again at school entry. Although antibody levels decline after the primary series in infancy, there is an excellent response to the booster at school entry and antibody levels persist at least until age 15, when another boost results in rapid and high increase in antibody. In the USA, the primary series is 2, 4 and 6 months and an additional boost is given at 18 months of age, resulting in another antibody peak. However, by school entry, levels have fallen close to those seen in the UK without the booster in the second year of life. Again, the response to a further booster in later childhood (e.g. 4–8 years) is excellent, and by the adolescent years the antibody profiles in both countries are similar. While the booster at age 18 months may give higher protection to the toddler and preschool age group, both schedules give good protection to schoolchildren and lay the foundation for long-lasting protection after a booster in adolescence.

The few data available on the duration of immunity following immunization in the Expanded Programme on Immunization (EPI) schedule, often have caveats such as the design of the study (cross-sectional or longitudinal), the type of assay performed, and whether or not the data analyses are appropriate, and whether ages at vaccination or duration since last vaccination are correct. Consequently, it is very difficult to interpret the data on duration of antibody levels following immunization under the EPI schedule. Data from the United Republic of Tanzania demonstrated that antitoxin levels  $\geq 0.1$  IU/mL were observed in 97% and 54% of children aged 1–5 and 6–15 years respectively (Aboud et al. 2000).

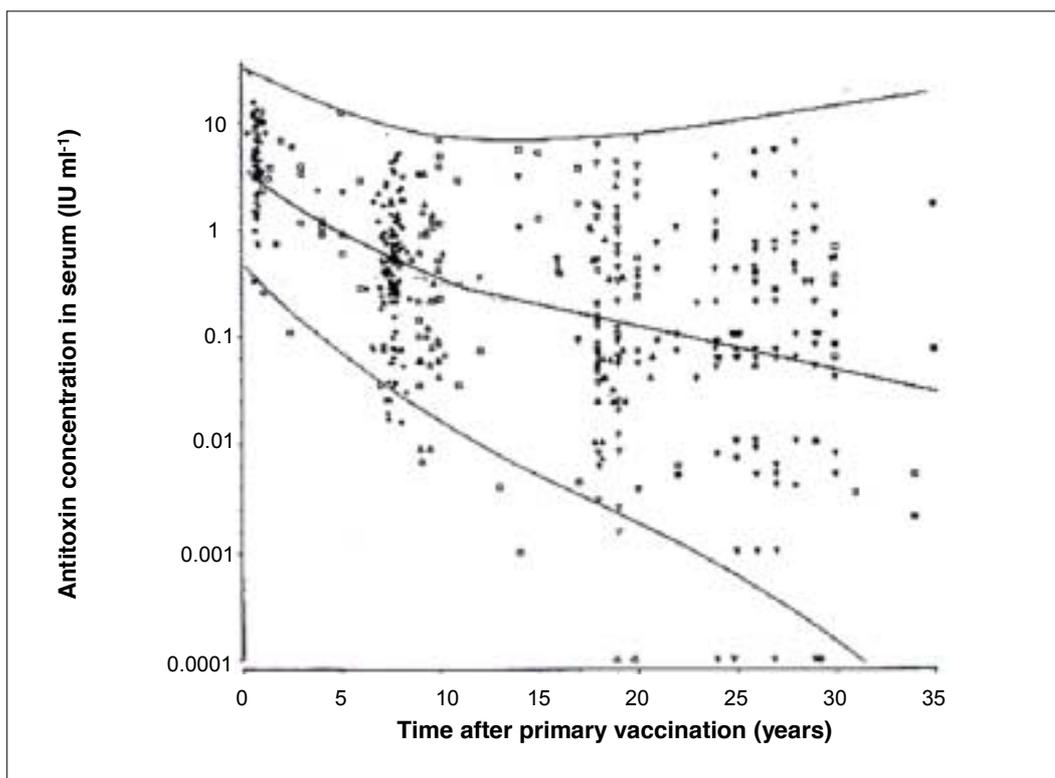
**Figure 4. Response to tetanus immunization following various schedules**



Sources: Data combined from Ramsay et al. 1993 (DTwP/IPV/Hib vaccine); Burrage et al. 2002 (DT or Td); Vergara et al. 2005 (DTaP/IPV vaccine); Swartz et al. 2003 (DTwP/IPV vaccine); Lin et al. 2003 (DTaP/IPV/Hib); Scheifele et al. 1999 (DTwP/IPV/Hib). Assay: ELISA except for Ramsay et al = RIA.

Data from studies performed in Denmark and elsewhere, not only demonstrate the longevity of the immune response to a primary series of tetanus toxoid vaccinations, but also the persistence of immune memory as evidenced by the response to revaccination many years later (Simonsen et al. 1987a; Volk et al. 1962; Simonsen et al. 1987c; Trinca, 1974; Turner et al. 1954; Simonsen et al. 1984). Analysis of the antibody levels of 439 subjects who had received three or four doses of tetanus toxoid and no revaccination revealed that levels were above 0.1 IU/mL (by ELISA) up to 25 years since last immunization (Figure 5). The authors concluded that primary immunization in infancy (three doses) gives approximately five years protection and that revaccination within five years of the last dose induces immunity for approximately 20 years. Data from a cross-sectional study in the Kingdom of the Netherlands (de Melker et al. 2000), where six doses of tetanus toxoid are given in childhood, with the last at age eight or nine years, also demonstrated that at approximately 20 years after the last dose the geometric mean antibody level was 0.44 IU/mL (ToBI assay).

**Figure 5. Duration of protection in Danish subjects following three or four doses of tetanus toxoid and no revaccination**



*Immunization Schedule relevant to study participants:*

*Infancy – either 3 doses of DT (12 Lf TT) at 5, 6 and 15 months or 4 doses DTP (7Lf TT) at 5, 6, 7 and 15 months, or 3 doses DTPol (Polio) (7Lf TT) at 5, 6 and 15 months.*

*Adults – 3 doses of TT (12 Lf) at 0, 1 month and 1 year apart.*

*Each data point represents an individual immunized with one of the described immunization schedules (n = 469).*

Historically, boosters were recommended every 10 years in the USA due to concerns about shorter-lived immunity with fluid tetanus toxoid rather than adsorbed, and that potency of either preparation varied (Levine et al. 1966). Furthermore, booster responses may vary individually and a pronounced dispersion of antibody levels can be expected with time following a primary series of immunizations (Figure 5); hence regular boosters were used to maximize protection for a high proportion of the population. The need for boosters every 10 years has been questioned because of the few numbers of cases and deaths in people who have received a full primary series of three or more doses, and also because of the literature illustrating that the duration of immunity is likely to exceed 10 years — a duration of 20 to 30 years has been suggested (De Melker et al. 2000; Simonsen et al. 1987a). Many countries, including the USA (Centers for Disease Control and Prevention, 1991), still recommend boosters every 10 years, whereas some countries, such as the UK, do not recommend any further doses following the five doses received as a child and adolescent, except, if appropriate, in wound management (UK Department of Health, 2006).

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The magnitude of the response to a booster dose of tetanus toxoid can depend on the time since last vaccination, and circulating antibody level. It has been widely reported that the higher the pre-booster antibody titre, the lower the relative increase in antitoxin response to immunization (Danilova et al. 2005; Levine et al. 1966). The clinical relevance of this observation is that boosting an individual with high antitoxin levels does not provide additional short-term or long-term protection. Therefore, immunization schedules need to be appropriately spaced to provide the optimal regime for booster vaccinations. Furthermore, if the schedule of primary or booster immunizations is interrupted, there is no requirement to re-start the primary series as it is likely that the response to the next dose in the series will sufficiently boost the levels of antitoxin.

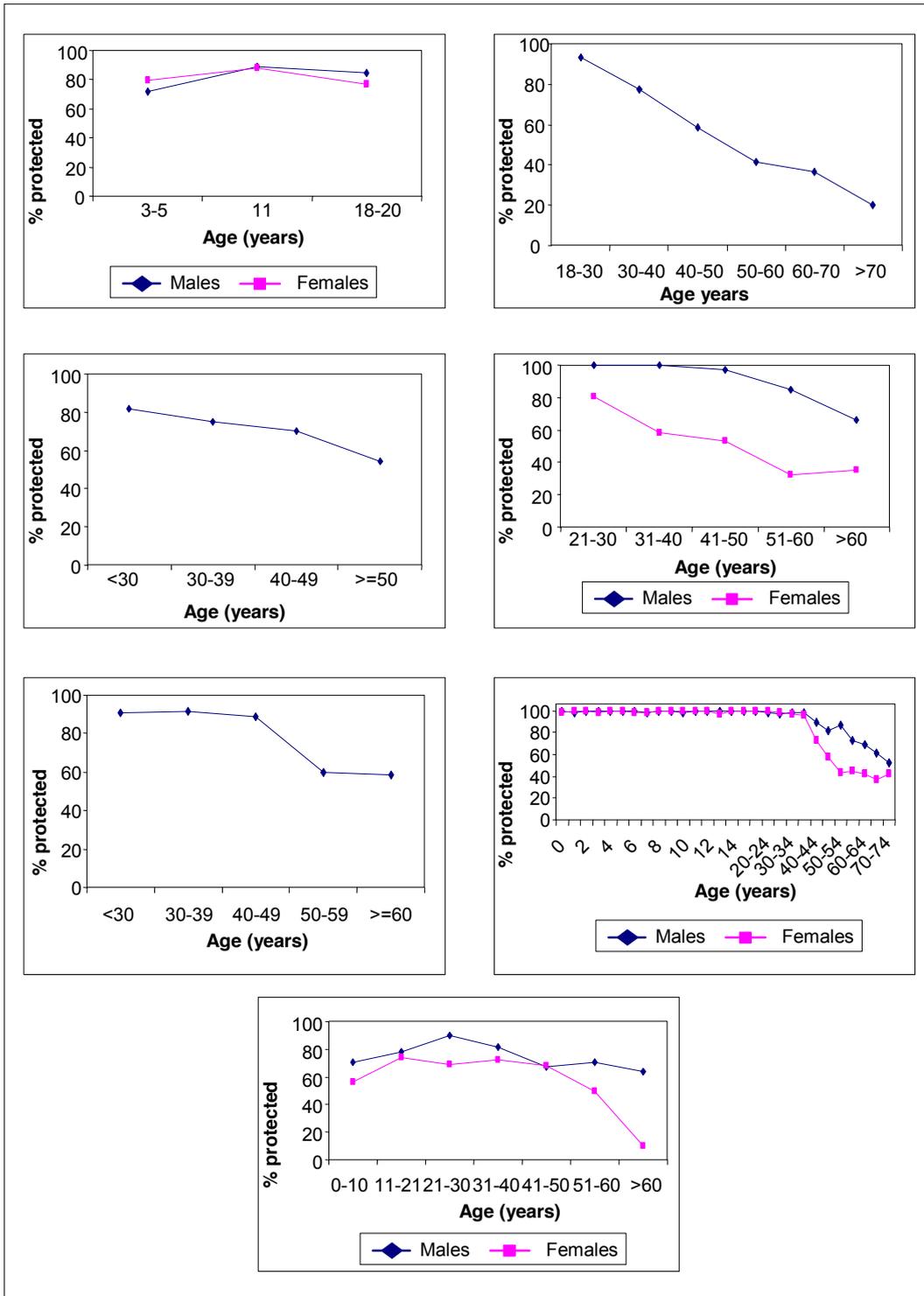
The kinetics of the response to a tetanus booster is of importance, both to predict protection of neonates after administration of boosters to pregnant women, and because it is recommended as a part of management of patients with tetanus-prone wounds. The median period of incubation to onset of tetanus has been reported as seven days [range 0–112 days] (Pascual et al. 2003). A measurable increase in antibody titre following a booster dose has been detected after four days (Turner et al. 1954; Simonsen et al. 1987c) but in general it takes six to seven days to reach substantial antitoxin levels (Looney et al. 1956; McCarroll et al. 1962; Turner et al. 1954). It is thought maximum levels are reached by two weeks post-booster (Volk et al. 1962; Evans, 1943) with one study demonstrating peak antibody levels at 11 days (Simonsen et al. 1987c). Hence, it is possible that administration of a tetanus booster as part of wound management will not contribute to the prevention of a current tetanus infection in incubation if antitoxin levels are low, but will provide long-term protection against future tetanus episodes.

In summary, three DTP doses in infancy will give three to five years of protection and there are limited data suggesting this may persist up to seven years (Volk et al. 1962); a further dose/booster (e.g. in early childhood) will provide protection into adolescence, and one or two more boosters will induce immunity well through adulthood — a duration of 20–30 years has been suggested. Booster responses can still be elicited after intervals of 25–30 years, demonstrating the persistence of immunological memory.

### **5.3 Tetanus immunity in different age and sex groups**

Serological surveys of anti-tetanus antibody levels in different age groups provide an understanding of the pattern of immunity and can show the effect that different vaccination schedules have on providing population immunity. Differences between men and woman are also highlighted, due to either vaccination in the military (mainly males), or countries where tetanus immunization occurs during pregnancy. In general men have higher antibody levels due to immunization during military service; however data from the Republic of India (Misra & Rao, 1988) demonstrated the impact immunization of women during pregnancy has on the antibody profile of females.

**Figure 6. Tetanus immunity in men and women in different age groups**

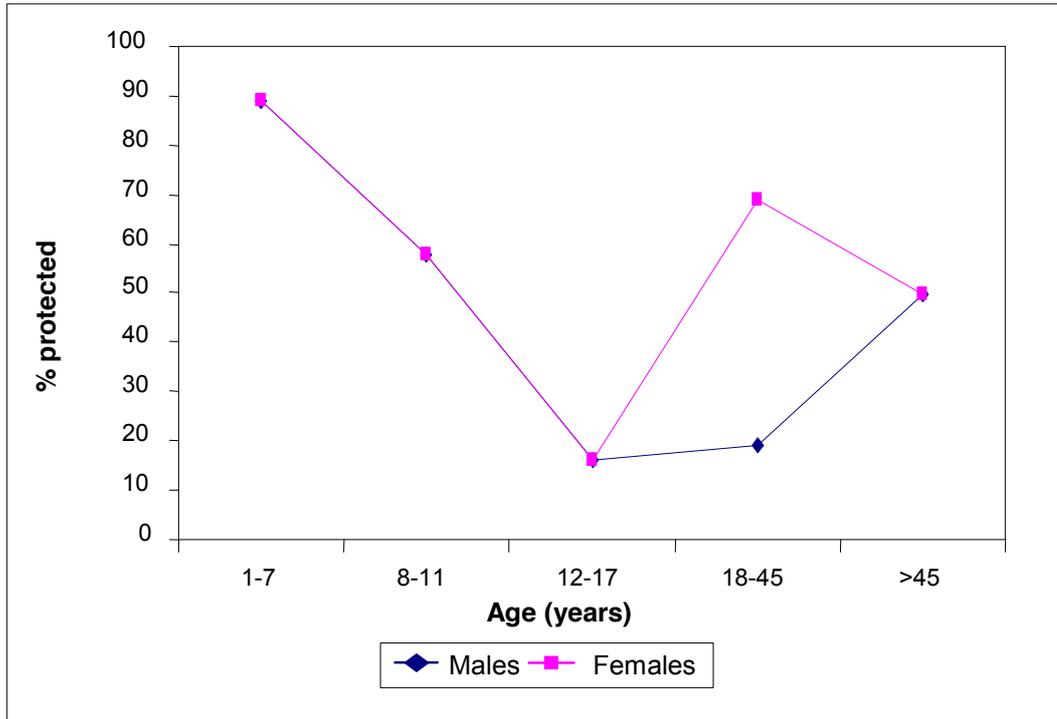


	Country	Reference	Assay	Protective Threshold (IU/mL)	Schedule
a)	Italy	Stroffolini et al. 1997	PHA	0.1	3, 5, 11 months, 5–6 years (+ every 10 years)
b)	Turkey	Ergonul et al. 2001	ELISA	0.01	2, 3, 4, 18 months, 6 & 13 years
c)	Germany	Stark et al. 1999	ELISA	0.1	3, 4, 5, 12–18 months, 3 & 8 years
d)	Greece	Symeonidis et al. 2003	ELISA	0.1	2, 4, 6, 18 months, 4–6 years (+ every 10 years)
e)	Canada	Yuan et al. 1997	ELISA	0.15	2, 4, 6, months, 4–6 years (+ every 10 years)
f)	Netherlands	De Melker et al. 2000	ToBI	0.01	3, 4, 5, 11 months, 4–9 years
g)	Egypt	Redwan et al. 2002	ELIS	0.15	2, 4, 6, 18 months

Figure 6 highlights data with trends of higher antibody levels in younger age groups and a decline in antibody levels as age increases. The difference between males and females is not as apparent, except for the data from the Hellenic Republic of Greece reported in 2003 and the older age groups in the Kingdom of the Netherlands from 1996. The majority of the data comes from developed countries with established immunization programmes, with high coverage during childhood and adolescence and variable booster coverage in adulthood (given with wound care or as part of routine prevention). A cross-sectional study from the Republic of Kenya (Figure 7) is of interest because the data illustrate tetanus immunity in a population where the EPI schedule is used. The EPI reached the district in which the study took place in 1983, and there was an initial campaign to immunize young children and pregnant women. Antibody levels are high for children aged one to seven years who would have been immunized under the EPI schedule or catch-up campaign, but lower in older children and adolescents (8–17 years). There is a rise in the antibody levels in females of childbearing age due to the immunization programme in place. These data highlight the fact that serosurveys require knowledge of the immunization programme, and coverage for each birth cohort to allow correct interpretation.

The serological surveys also illustrate the potential for appropriately scheduled primary series and boosters to provide high antibody levels for women throughout childbearing age. The data shown in Figure 6 and other serological surveys reported in the literature (Maple et al. 2001; McQuillan et al. 2002) demonstrate that consistent antibody levels remain for approximately 80% of women, from a young age until the age of approximately 40 years before decreases in antibody levels are observed. This does vary by country and immunization schedule, but suggests that a complete primary series of immunizations and subsequent boosters in childhood and adolescence provides protective antibody levels well into adulthood, protecting women (and their newborns) throughout their childbearing years.

**Figure 7. Tetanus immunity in men and women in different age groups in Kenya**



*Protective Threshold: 0.01 IU/mL.  
Immunization schedule: 6, 10, 14 weeks.*

#### **5.4 Factors influencing the response to tetanus toxoid**

Two conditions that may influence the immune response to tetanus toxoid are malaria and human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS). In many areas where neonatal tetanus is still common, these infections are also widespread.

The response of malaria-infected pregnant women to tetanus toxoid immunization is similar to that of non-pregnant healthy adults (Brabin et al. 1984). In children, two studies reported a decreased response following one or two doses of tetanus toxoid in children with parasitaemia from an acute attack of malaria compared to non-parasitaemic controls (Edsall et al. 1975; Greenwood et al. 1972). Chemoprophylaxis for malaria can be given without any impairment of the antibody responses to immunization in children (Gilles et al. 1983; Monjour et al. 1982; Rosen & Breman, 2004). Dietz et al (1997) concluded following a review of available literature, that concurrent malarial infection may decrease the immune response to tetanus toxoid but that complete evaluation of the impact of concurrent malarial infections on the response to tetanus toxoid requires analysis of infected and non-infected individuals; however withholding chemotherapy from the infected group would be unethical.

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Because inactivated vaccines are safe for use in immunocompromised individuals, the main concern in HIV-infected persons given TT is the effectiveness of immunization. Persons with symptomatic HIV infection have several immunological abnormalities, including hypergammaglobulinemia, decreased CD4 lymphocytes, poor T-lymphocyte response to mitogen stimulation, and altered humoral immunity. In such persons, abnormal primary and secondary antibody responses may result in decreased efficacy of immunization. Abnormalities of the immune response become more severe with advancing disease (Blanche et al. 1986). HIV infection interferes with antibody responses to antigen encountered after infection has occurred, but affects the antibody responses of lymphocytes “educated” prior to infection less severely (Borkowsky et al. 1987).

HIV-positive children given three doses of tetanus toxoid (DTP) at 6, 10 and 14 weeks had similar proportions protected at nine months of age as HIV-negative children, 95.8% and 94% respectively (Ryder et al. 1993). Administration of three doses at 2, 4 and 6 months resulted in a protective titre in 100% of HIV-positive children, as assessed by dot blot with a protective threshold of > 0.01 IU/mL (Borkowsky et al. 1992). However, there are reports of impaired antibody responses to tetanus toxoid following primary immunization (Blanche et al. 1986; Barbi et al. 1992). Moss et al (2003) concluded that 40%–100% of HIV-infected children develop protective levels of tetanus antitoxin following primary immunization in infancy. HIV-infected children appear to respond well to booster immunization with between 74%–90% reported to have protective antibody levels following a booster dose at various ages and times since primary series (Borkowsky et al. 1992; Rosenblatt et al. 2005; Melvin & Mohan, 2003).

Limited data are available on primary immunization of HIV-infected adults but the response to a booster dose induces protective levels (Kurtzhals et al. 1992); however the response tends to be lower than in uninfected controls (Kroon et al. 1995; Dieye et al. 2002; Bonetti et al. 2004). The duration of circulating antitoxin following primary or booster immunization in HIV-positive individuals is uncertain (Talesnik et al. 1998; Moss et al. 2003).

Tetanus toxoid, as a monovalent vaccine or as a component of combined vaccines, is recommended for HIV-infected children or adults, regardless of the presence or absence of symptoms of AIDS, and for individuals with malarial infection, as most vaccine recipients, both children and adults, appear to achieve protective antitoxin levels.

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# 6. Placental passage of tetanus antitoxin

## 6.1 The placenta as a selective organ

Tetanus antitoxin transferred from immunized mother to fetus provides transient protection of the newborn infant from tetanus. The human placenta regulates the transfer of antibodies from mother to fetus in a selective manner; transplacental transfer is restricted to IgG immunoglobulin. Fetal IgG antibody levels rise progressively from the fourth month of pregnancy until term. At birth, the infant usually has a total tetanus antibody concentration equal to, or sometimes higher than, the mother. Early studies found that the tetanus antitoxin levels in cord serum and maternal serum were usually equal, although in 20% to 30% of cases the cord serum had a lower titre than the maternal serum. This may be attributed to the presence of only IgG in neonates, although this observation is dependent upon the assay used, as the HA will detect IgG and IgM in mothers and IgG in the newborns. It was observed that the cord/maternal ratio of tetanus antibodies is higher in European than in African settings (Gendrel et al. 1990a, 1990b). This may be linked to high immunoglobulin levels in African mothers exposed to multiple antigenic stimuli.

## 6.2 Influence of interval between TT doses and between the last dose and delivery on the amount of antitoxin transferred to the fetus

The ratio of antitoxin in maternal serum to antitoxin in cord serum depends on the intervals between doses of tetanus toxoid and the interval between the last dose and delivery. Longer intervals between doses of tetanus toxoid in the initial series increase the height and duration of the immune response (Table 2). Long intervals between doses of toxoid are best for achieving the optimal immunological results. However, in reality, pregnant women in developing countries often report to health centres, and are immunized for the first time, when pregnancy is already advanced (Figure 8). Often, the second dose of tetanus toxoid is given just before the delivery, which diminishes the possibility of effective transfer of a significant amount of antibody from the mother to the fetus. The cord/maternal ratio of tetanus antibodies increases as the interval between the second dose and delivery is prolonged (Stanfield et al. 1973). These data strongly support the policy of starting immunization as early as possible in the pregnancy, to ensure adequate intervals between doses and between the second dose and delivery.

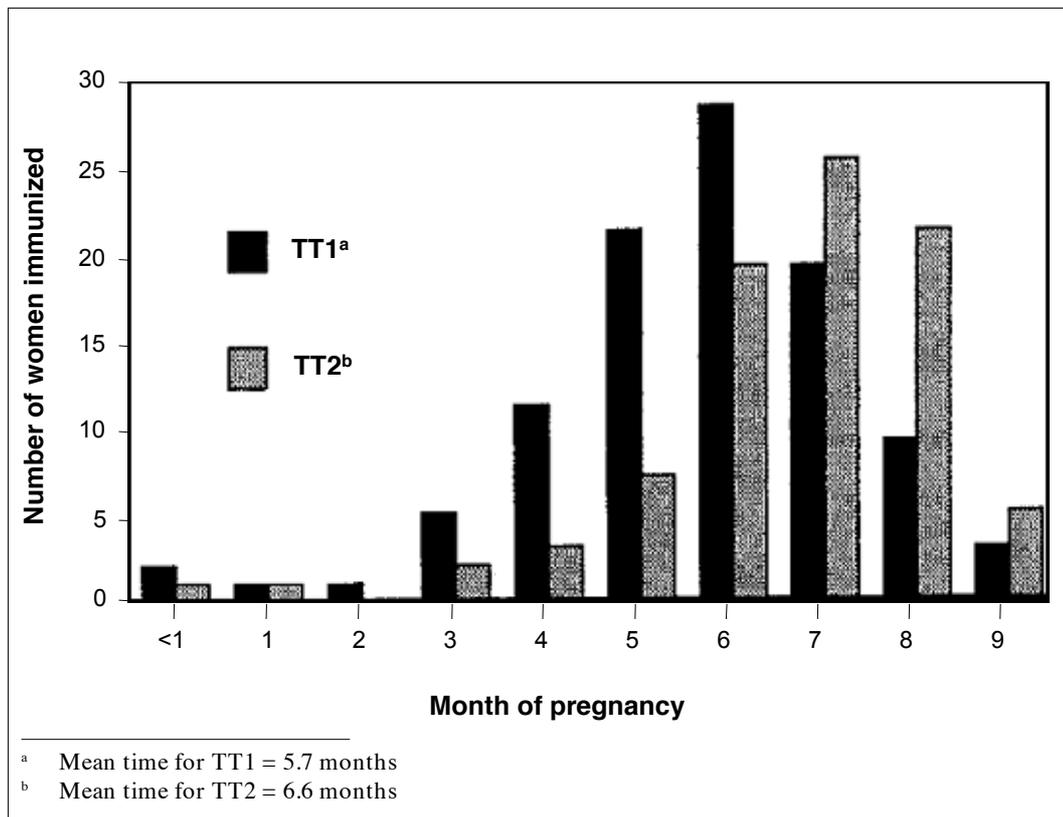
Nonetheless, even if women first present to health services late in pregnancy, the opportunity should be taken to administer primary (or booster) immunization(s) if indicated, in order to contribute to long-lasting immunity, and protection in subsequent pregnancies.

**Table 2. Tetanus antitoxin level in cord sera of neonates whose mothers were immunized with two doses of tetanus toxoid administered at different intervals**

Interval between toxoid doses (weeks)	No. of samples tested	% distribution of antibody levels (IU/mL) in cord sera		
		>0.01	>0.1	>1.0
4 to 8	238	70.6	37.0	8.4
9 to 12	210	81.1	62.4	15.7
13 to 16	133	92.5	71.4	22.6
Over 16	142	90.8	73.9	39.4

Source: Galzaka, 1993 (Original data from Dhillon & Menon 1975).

**Figure 8. Time of TT immunization during pregnancy, Lagos State, Nigeria**



Source: Galzaka, 1993.

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### 6.3 Factors influencing the placental transfer of antitoxin

The results of studies on the effect of placental malaria infection on trans-placental transfer of tetanus-specific antibody have varied. In a study in the Republic of the Gambia in 1997, in which malaria infection was assessed by measuring parasitaemia in mothers' blood (Okoko et al. 2001), no effect was observed on the transfer of tetanus antibody. Similar findings were reported in a study performed in the Republic of Malawi where placental malaria was assessed on blood samples collected from a deep incision on the maternal side of the placenta (de Moraes-Pinto et al. 1998). However, two studies that took placental biopsies to determine placental malaria infection, showed a reduction in the transfer of tetanus antibodies. In a study from Papua New Guinea, approximately 10% of infants of women with heavy placental parasitization failed to acquire a protective tetanus antibody level despite protective levels in the mothers (Brair et al. 1994). More recent data from Kenya has demonstrated a reduction in transfer of tetanus antibodies and lower antibody levels in neonates associated with active chronic and past placental malaria infections, but not active acute malaria infection (F. Cutts, personal communication).

The placental transfer of immunoglobulins has been shown to be reduced by maternal HIV infection (de Moraes-Pinto et al. 1996; de Moraes-Pinto et al. 1998; Scott et al. 2005) including the transfer of tetanus antibodies (de Moraes-Pinto et al. 1996).

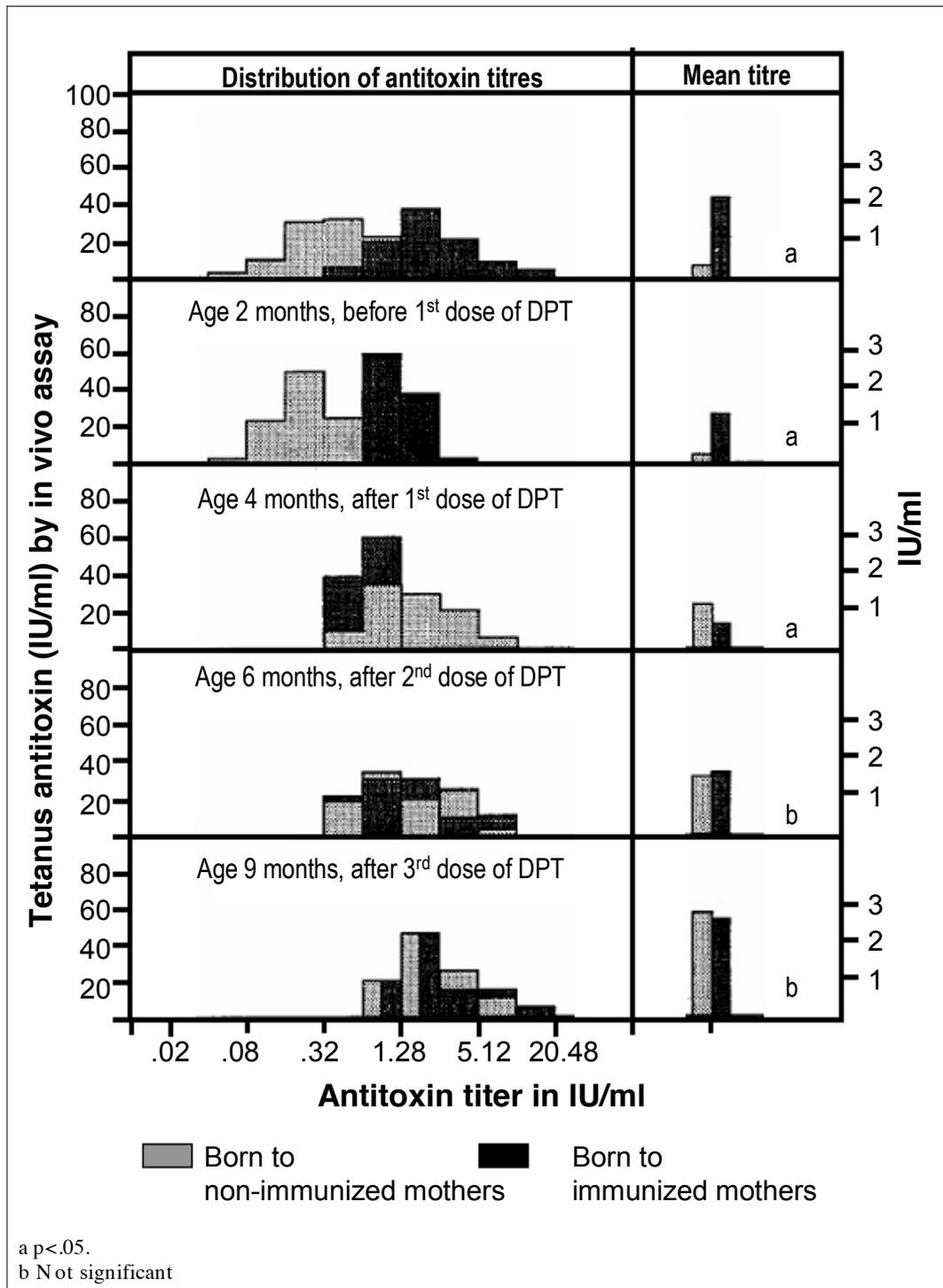
### 6.4 Interference between passive antibodies and development of active immunity

The rate of decrease of tetanus antitoxin during the neonatal period (Kryl et al. 1964; Sangpetchsong et al. 1985) is similar to that for antibodies against *Neisseria meningitidis* group A, *Haemophilus influenzae* type b (Hib) and *Streptococcus* group B induced by polysaccharide vaccines given to mothers during pregnancy (Amstey et al. 1985; Baker et al. 1988; McCormick et al. 1980). After one month, about 80% of antitoxin transferred from the mother is still present in the circulation of the newborn.

With an increasing proportion of women immunized with tetanus toxoid, more and more infants will have high levels of passively-acquired tetanus antitoxin. Such passive immunity could suppress the development of active immunity following early administration of DTP vaccine. Results of one study showed some interference between passive immunity acquired from mothers immunized three times during pregnancy, and active immunity following two doses of DTP vaccine administered at two to six months and three to seven months (Kryl et al. 1964). The interference was accentuated in infants who had cord serum titres above 0.1 IU/ml. Data from the Kingdom of Thailand on infants immunized at 3, 4, and 6 months of age show a suppressive effect of passive immunity after the first dose of DTP vaccine, but not following the two subsequent doses (Figure 9). By contrast, in the Republic of the Philippines, tetanus antibody levels in children at age six weeks were positively correlated with the number of TT doses received by the mother during pregnancy, while the infant's antibody levels achieved after the primary series of three doses of DTP were negatively correlated with the number of doses received by the mother.

Although this suggests that high levels of transplacentally-acquired antibody can reduce the response to DTP, the authors note that the clinical and public-health significance of this is not known, since all children maintained titres considered protective up to the age of 10 months, when a booster dose was given (Nohynek et al. 1999).

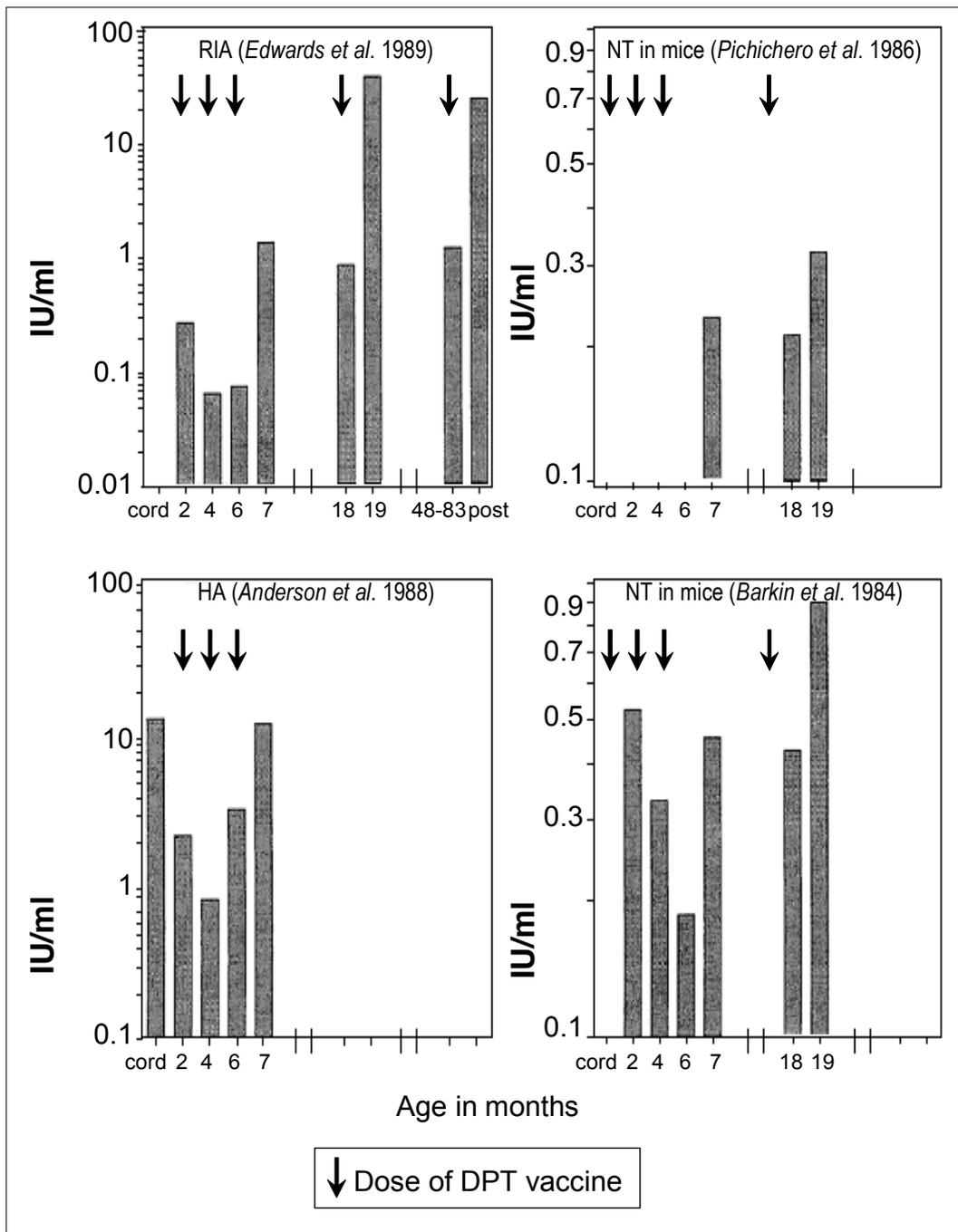
**Figure 9. Tetanus antitoxin titres in DTP-immunized infants whose mothers were immunized or not immunized against tetanus**



Source: Galazka, 1993 (Original data from Sangpetchsong et al. 1985).

In developed countries, the majority of women of childbearing age are immune against tetanus. In the USA, the mean tetanus antibody level in cord serum is high, exceeding 10 IU/ml, when measured by the haemagglutination test (Anderson et al. 1988). With a half-life of about one month, the antibody level determined by the neutralization test declines to 0.3–0.5 IU/ml by the age of two months, when the first dose of DTP vaccine is administered (Barkin et al. 1984; Edwards et al. 1989). This level of passive immunity interferes with the first dose(s) of DTP, but the third dose of DTP exerts a strong antigenic stimulus (Figure 10).

**Figure 10. Tetanus antibody levels in children immunized in the USA with DTP vaccine at 2, 4, 6, 18 months and 4 to 6 years of age**



Source: Galazka, 1993

# 7. Effectiveness of tetanus toxoid

## 7.1 How effective is tetanus toxoid?

The efficacy (as measured in randomized, controlled clinical trials), and effectiveness (as measured in observational studies under field conditions), of tetanus toxoid has been convincingly demonstrated in many field trials and in hospital-based studies. A double-blind, controlled field trial in a rural area of the Republic of Colombia conducted in the 1960s showed that adsorbed tetanus toxoid administered to women of childbearing age provided substantial immunity against neonatal tetanus. A control group had a neonatal tetanus mortality rate of 78 per 1000 live births, whereas no neonatal tetanus cases occurred in babies of mothers given two or three doses of tetanus toxoid (Newell et al. 1966, 1971). A reduction in neonatal tetanus mortality following the implementation of programmes to immunize women of childbearing age, and especially of pregnant women, has also been observed in multiple countries, and published for Bangladesh (Black et al. 1980; Rahman et al. 1982), the Republic of Haiti (Berggren et al. 1983), the Republic of Mozambique (Cliff 1985a & b), the Republic of Namibia (EPI, 2002), the Republic of South Africa (Vandelaer et al. 2003), the Democratic Socialist Republic of Sri Lanka (EPI, 1982) and the Republic of Zimbabwe (EPI, 2001). Surveys of neonatal tetanus mortality also provide data about mortality rates for children born to vaccinated and nonvaccinated mothers; these data are useful in assessing tetanus toxoid vaccine effectiveness. In most studies, tetanus toxoid vaccine efficacy ranged from 80% to 100% (Table 3).

**Table 3. The neonatal tetanus mortality rate and the clinical efficacy of two doses of tetanus toxoid (TT2) in preventing neonatal tetanus, as determined by community-based surveys in six countries**

		Neonatal tetanus mortality rate/1000 live births				
Country	No. of live births surveyed	Overall rate	Rate for cases born of mothers...		Efficacy of TT2 (%)	Reference
			Immunized	Non-immunized		
Burma	6 000	6.8	1.5	11.1	86	Stroh et al. 1986
Egypt	12 000	4.8	0.8	6.0	88	EPI, 1987
Ethiopia	2 010	4.5	0	5.8	100	Maru et al. 1988
India	4 344	3.5	1.3	6.1	79	Kumar et al. 1988
Indonesia	4 971	10.7	1.4	12.5	89	Arnold et al. 1986
Islamic Republic of Iran	2 655	6.0	0	9.2	100	Sadeghi-Hasanbadi, 1987

Source: Galazka, 1993

## 7.2 Reported “failures” of tetanus toxoid immunization

Tetanus toxoid is one of the most reliably immunogenic antigens used in current vaccines, although, as mentioned above, it is not 100% effective. Clinical cases of tetanus have been reported despite previous tetanus toxoid immunization (Table 4). There is no clear pattern associated with these cases with respect to immunization. There are varied immunization histories, ranging from uncertain of any previous immunization, to hyperimmunised for the purpose of production of tetanus immune globulin.

**Table 4. Tetanus cases and deaths reported in persons immunized with tetanus toxoid 1946–2000**

Reference	Tetanus toxoid immunization status	No. of cases	No. of deaths
Boyd, 1946	Primary series or incomplete	7	3
	Routine booster(s)	9	2
	Emergency booster	1	0
Hall, 1948	Uncertain	1	1
	Primary series	1	0
	Emergency booster	1	0
Hedrick, 1953	Primary series	1	0
	Routine booster	1	0
Boyer et al. 1953	Primary series or incomplete	9	7
	One booster, 3 years previously	1	1
Long, 1954	Primary series	2	0
	Routine booster, 3 months previously	1	0
	Emergency booster	4	2
Moss et al. 1955	Routine booster	2	1
	Emergency booster	1	0
Christensen & Thurber, 1957	Primary series, 10 years previously	1	1
Peterson, 1965	Emergency booster	1	0
Spittle, 1973	Several boosters	1	0
Berger et al. 1978	Uncertain	1	0
	Primary series, 15 years previously	1	0
Baptist, 1984	Incomplete primary series	1	1
Passen & Andersen, 1986	Boosters 8 and 4 years previously	1	0
Crone & Reder, 1992	2 individuals had a dose 1 year prior, 1 individual had been hyperimmunized	3	1
Pryor et al. 1997	Primary series 2 boosters	1	0
Abrahamian et al. 2000	Uncertain	1	1
Lodha et al. 2000	Primary series and booster	2	0
Pascual et al. 2003	1 dose	10	0
	2 doses	0	0
	3 doses	2	0
	≥ 4 doses	18	1

Source: Galazka, 1993

Similarly, while the number of neonatal tetanus cases declines as maternal immunization coverage rises, the proportion of cases in newborns whose mothers had been immunized increases because TT is not completely effective. Table 5 shows that although neonatal tetanus occurs despite maternal immunization, the vast majority of cases occurred when mothers were either unvaccinated or inadequately vaccinated. In the Federal Republic of Nigeria, neonatal tetanus was observed in six infants whose mothers had received  $\geq 2$  doses of tetanus toxoid during the last pregnancy, and where the antibody levels of both mothers and infants were of a magnitude expected to confer protection (mean antibody level by ELISA: 0.70 and 1.02IU/mL for newborns and mothers, respectively) (de Moraes-Pinto et al. 1995). The reasons for the occurrence of neonatal tetanus in these infants are unclear, but could have included heavy tetanus spore contamination of the cord resulting in particularly large quantities of toxin production.

**Table 5. Tetanus toxoid (TT) immunization history of mothers whose infants developed neonatal tetanus (NT), based on hospital data**

Country	Reference	No. of NT studied	Maternal history: number of doses of TT			
			0	1	2	3
Angola	Grudeborn, 1987	199	188	0	11 <sup>a</sup>	0
Egypt	El-Sherbini, 1991	74	55	19	0	0
Egypt	Gad et al. 1986	324	324	0	0	0
India	Bildhaiya, 1983	74	73	0	0	1 <sup>b</sup>
India	Deivanayagam et al. 1991	19	13	0	3 <sup>c</sup>	3 <sup>c</sup>
India	Ghosh, 1990	30	21	5	4	0
India	Kumar et al. 1988	385	363	0	22 <sup>d</sup>	0
India	Mathure et al. 1980	50	50	0	0	0
India	Verma et al. 1989	76	49	5	12 <sup>e</sup>	10
Mozambique	Cliff, 1985	175	173	0	2	0
Nigeria	Einterz & Bates, 1991	237	234	"several"	1	0
Nigeria	Grange, 1991	419	411	8	0	0
Nigeria	Owa & Makinde, 1990	52	35	5	11	1
Nigeria	Oyedeyji et al. 1982	104	97	3	3	1

<sup>a</sup> Immunized during pregnancy with TT.

<sup>b</sup> Immunized in childhood with DTP vaccine.

<sup>c</sup> Out of three mothers who received 2 doses of TT the second dose was given in the 9<sup>th</sup> month of pregnancy in two mothers; out of three mothers who received 3 doses, the third dose was given in the 9<sup>th</sup> month of pregnancy in one mother.

<sup>d</sup> 22 mothers "fully" immunized.

<sup>e</sup> One mother received the second dose two days before delivery.

Source: Galazka, 1993

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There are several explanations for reports of neonatal tetanus cases occurring in infants of women claiming to be immunized.

1. Inaccurate immunization history. Maternal immunization status is often based on verbal history, rather than written documentation. In many countries written records are not given to mothers, or they do not retain records they are given. In some countries pregnant women receive many injections unrelated to tetanus toxoid immunization, and this may lead to confusion about tetanus toxoid immunization status. Discrepancies in history of tetanus toxoid immunization were observed in two reports where the level of those immunized varied between documented evidence and verbal history compared to the levels of seropositive mothers (EPI, 1996a), and where 45% of mothers who said they had received no tetanus toxoid dose in last pregnancy or earlier were considered seropositive (Deming et al. 2002).
2. Receipt of vaccine late in pregnancy. Many women report late in pregnancy for antenatal care (refer to section 6.2). Consequently, if immunization is required (see section 10.0 and Table 7), completion of the recommended number of doses is often too close to delivery for the mother to develop and transfer sufficient antitoxin for adequate newborn protection (although the vaccine will contribute to long-term immunity and help to protect neonates in subsequent pregnancies).
3. Low potency vaccine. The tetanus toxoid itself may not be potent due to problems of manufacture, storage (e.g. freezing) or transport. A review by Dietz et al (1997) reported reduced potency with locally-manufactured tetanus toxoid. Hlady et al (1992) reported no potency in three consecutive lots of vaccine locally produced in Bangladesh.
4. Poor maternal immune response. In most studies performed in developing countries, two doses of tetanus toxoid stimulated the development of tetanus antibody levels considered protective in at least 80% of women, with additional doses protecting up to 98% of recipients. Some mothers, however, may have an antibody response below the protective level (“poor responders”).
5. Inadequate placental transfer. Data are available that suggests in areas where mothers’ immunoglobulin levels are excessively high due to continued multi-antigenic stimulation, placental transfer of antibodies may be less efficient, leaving the newborn inadequately protected (Gendrel et al. 1990b). Some, but not all, studies also show reduced placental transfer in women with malaria and/or HIV infection (see section 6.3).
6. Excessive toxin exposure. The load of tetanus toxin produced in a heavily contaminated umbilical cord stump may be so large as to overwhelm the modest immunity transferred from mothers immunized with only two doses of tetanus toxoid. It is important to note that the level of antibodies required to neutralize toxin is dependent upon the amount of toxin exposure; the quantity of toxin large enough to overwhelm the protection afforded by 0.01 IU/mL is currently unknown. Furthermore, the levels of umbilical cord exposure to toxin and the practices which lead to this exposure are likely to differ in at-risk sub-populations.

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## 8. Safety of tetanus toxoid

Tetanus toxoid is one of the most extensively used antigens in vaccinations. The acceptance of tetanus immunization demonstrates its known effectiveness and excellent safety profile. Mild local reactions are relatively common following immunization with tetanus toxoid, but severe adverse events are extremely rare. There are many factors which influence the frequency and severity of adverse events, such as the number of prior doses, level of existing antibodies, route of injection, and the presence of other antigens in the preparation (Wassilak et al. 2004).

Local reactions are the most common adverse event associated with tetanus immunization. It is believed that pre-existing antitoxin forms complexes with the deposited toxin, known as the Arthus reaction, which results in local swelling and pain (Edsall et al. 1967; Eisen et al. 1963; Levine & Edsall, 1981). The rate of local reactions is reported to increase with an increasing number of doses (Myers et al. 1982; Relihan, 1969; White, 1973). However, recent reports of combination vaccines containing tetanus toxoid have found an acceptable safety profile with regard to local reactions in various age groups (Knutsson et al. 2001; Saenger et al. 2005; Huang et al. 2005; Mallet et al. 2004). More severe local reactions characterized by marked swelling occur in fewer than 2% of vaccine recipients (Relihan, 1969; Sisk & Lewis, 1965).

Systemic reactions such as fever, headache and malaise have been reported after tetanus immunization (Macko, 1985; Sisk & Lewis, 1965; Levine & Edsall, 1981; White, 1973) with approximately 10% of adults reporting a systemic reaction following administration of a Td vaccine containing tetanus toxoid (Lloyd et al. 2003). In infants, fever and irritability are reported at higher rates, approximately 20%–25% following administration of a combination vaccine containing tetanus toxoid (Knutsson et al. 2001; Mallet et al. 2004).

There have been reports of brachial plexus neuropathy occurring following tetanus immunization (Quast et al. 1979; Holliday & Bauer, 1983; Tsairis et al. 1965; Beghi et al. 1985; Hamati-Haddad & Fenichel, 1997). In 1994, the United States Institute of Medicine (IOM) concluded that a causal relationship between tetanus immunization and brachial plexus neuropathy is likely, estimating that 0.5–1 cases per 100 000 TT vaccine recipients were attributable to tetanus toxoid (IOM, 1994). It has been suggested that this conclusion may be an overestimation due to the limited nature of the data reviewed to reach the conclusion (Wassilak et al. 2004).

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Guillain-Barre Syndrome (GBS) following tetanus toxoid is rare, but a causal relationship may exist (IOM, 1994; Newton & Janati, 1987). In one report an individual had GBS on three separate occasions following tetanus immunization (Pollard & Selby, 1978). However, two studies suggest that there is no causal relationship between GBS and tetanus toxoids. One study observed only one case of GBS six weeks post-immunization in adults (> 18 years of age) following an estimated 1.2 million doses of tetanus toxoid-containing vaccines, whereas two cases were expected by chance alone (Tuttle et al. 1997). In a study of 0.7 million children of pre-school age, three cases of GBS were expected following immunization with DTP by chance, but only two were reported (Rantala et al. 1994). The United States IOM has estimated that the incidence of GBS following tetanus immunization is 0.4 per million doses (IOM, 1994).

Anaphylactic reactions to tetanus toxoid are rare (Wassilak et al. 2004; IOM, 1994; Zalogna & Chernow, 1982; Ratliff & Burns-Cox, 1983). Early reports of anaphylaxis were believed to be due to the presence of sensitizing agents in the vaccine preparations (Galazka, 1993; Wassilak et al. 2004). A recent review of anaphylaxis associated with childhood vaccines (Bohlke et al. 2003) found four cases of anaphylaxis associated with tetanus toxoid containing vaccines in approximately 2 million doses administered. Since the initiation of vaccine adverse event reporting to the CDC began in 1978, no deaths caused by anaphylaxis were reported during a period (1978–1996) in which more than 80 million doses of DTP had been administered (Advisory Committee on Immunization Practices (ACIP), 1996).

Tetanus toxoid is considered safe in pregnant women. There is no convincing evidence of risk to the fetus from immunizing the pregnant women with tetanus or diphtheria toxoids (ACIP, 1991). Immunization of women during pregnancy is a safe and effective strategy in the effort to achieve the goal of maternal and neonatal tetanus (MNT) elimination.

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## 9. Combination vaccines and concomitant vaccine use

Tetanus toxoid has been combined with diphtheria toxoid, pertussis vaccines and *Haemophilus influenzae* type b (Hib) conjugate vaccines for many years without causing any increase in adverse events or compromising the response to tetanus. The Hib conjugate comprises Hib-polyribosylribitol phosphate (PRP) covalently linked to tetanus toxoid. Studies have shown that maternal antibodies to tetanus antitoxin do not interfere with the response to Hib conjugate vaccines (Kurikka et al. 1996; Nohynek et al. 1999) and that simultaneous administration of DTP and Hib conjugate does not increase the frequency of common adverse events (Holmes et al. 1993). Hib conjugate vaccines cannot however replace the need for primary tetanus toxoid immunization (Carlsson et al. 1994).

The repertoire of combination vaccines that include tetanus toxoid is expanding. Additional antigens being added include Hepatitis A and B, inactivated polio and meningococcal serogroup C, with various vaccines already licensed or undergoing clinical trials. In 1996 WHO proposed the use of a DTP-Hepatitis B combination vaccine to support the recommendation of including Hepatitis B vaccination in the EPI programme (EPI, 1996b).

The success of Hib conjugate vaccines has led to the application of the technology to other bacterial polysaccharide-based vaccines such as meningococcal, pneumococcal and *Salmonella typhi*. The development of these conjugate vaccines has raised concerns regarding impairment or interference of responses, and safety concerns (see Section 8), but there is also the potential to enhance responses to the combination of antigens administered.

Significant enhancement of the tetanus response has been observed in UK infants following concomitant administration of diphtheria, tetanus and whole-cell pertussis with Hib conjugate and a meningococcal group C tetanus toxoid conjugate (Table 6, and Kitchin et al. 2006). Augmentation of the tetanus response has also been observed when a five component acellular pertussis vaccine is used in place of whole-cell pertussis (Kitchin et al. 2006).

**Table 6. Effect of concomitant MCC-TT vaccine on response to Hib and tetanus in UK infants (2, 3, 4 month schedule) receiving DTwP/Hib**

Vaccines given	PRP GMC ug/ml	Tetanus GMC IU/ml
DTwP + PRP-T <sup>a</sup>	4.50 (3.21–6.32)	0.65 (0.54–0.77)
DTwP/PRP-T <sup>*</sup>	3.39 (2.48–4.66)	1.19 (0.98–1.44)
DTwP/PRP-T+MCC-TT <sup>**b</sup> (10Lfs in DTP + 30ug PRP-T+15ug MCC-T)	11.59 (9.3–14.5)	4.00 (3.3–4.8)

<sup>a</sup> PRP - polyribosylribitol phosphate

<sup>b</sup> MCC – meningococcal serogroup C conjugate

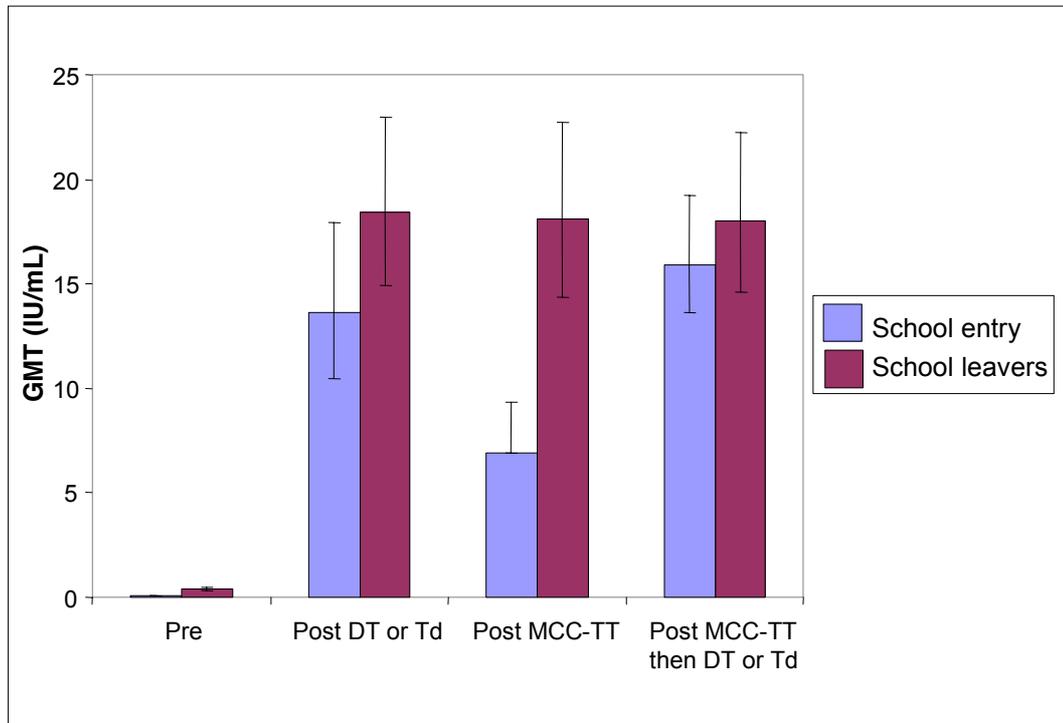
*Sources:*

\* *Begg et al., 1995*

\*\* *Richmond et al. 2001*

Data from the UK demonstrated the potential for conjugate vaccines containing tetanus toxoid as the carrier protein to enhance antibody responses to tetanus (Burrage et al. 2002). School entry-aged children (3.5 to 6 years) and school leavers (13 to 18 years) were found to make a substantial tetanus antibody response (geometric mean concentration (GMC) > 5 IU/mL) to a meningococcal serogroup C – tetanus toxoid conjugate (Figure 11). In school leavers, the tetanus antibody level was comparable following administration of either the meningococcal serogroup C – tetanus toxoid conjugate or a Td vaccine (Figure 11). Similarly, in the Philippines, children who had received a primary series of DTP at 6, 10 and 14 weeks showed strong boosting response to a dose of Hib-TT at age 10 months (Nohynek et al. 1999). This brings into question whether the response to tetanus toxoid used as a carrier protein in conjugate vaccines is sufficient or not to allow replacement of the routine boosting dose. The number of new combination vaccines used in countries using the EPI schedule will be limited, mainly due to cost. However, one interesting development of relevance, is the evaluation of a meningococcal serogroup A conjugate vaccine that is conjugated to tetanus toxoid and is planned for use in 1–29 year olds in the region of Africa known as the “meningitis belt” (Jodar et al. 2003b).

**Figure 11. Effect of meningococcal TT conjugates on the tetanus antibody levels induced following diphtheria-tetanus vaccines given at school entry or to school leavers**



Source: Burrage et al. 2002  
GMT – geometric mean titre

Suppression of tetanus antibody production has been observed following concomitant vaccine administration. Tetanus antibody levels after three doses of DTP were observed to decrease with increasing tetanus content of experimental pneumococcal conjugate vaccines given concomitantly (Dagan et al. 2004). The clinical relevance of this phenomenon is unclear with regard to inducing protective levels of antitoxin. Carrier-induced epitope-specific suppression is a phenomenon where inhibition of antibody response to a specific antigen is inhibited following prior immunization with the carrier protein. It is thought that this is due to expansion of carrier-specific B cells (Barrington et al. 1993). Reduced meningococcal serogroup C antibody responses to a conjugate vaccine with tetanus toxoid as the carrier have been observed in children following prior immunization with DT or Td vaccines (Burrage et al. 2002). It is clear that for any new combination vaccine the effect of prior or concomitant administration of proteins used in conjugate vaccines must be evaluated.

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# 10. Implications for immunization programmes

Much success has been achieved in the progress towards elimination of MNT since the World Health Assembly (WHA) first called for global neonatal tetanus elimination in 1989 (WHA, 1989). The estimated number of global neonatal tetanus deaths has declined from 787 000 in 1988, to 180 000 in 2002 (Vandelaer et al. 2003; EPI, 2006). In 1999, 57 (58 with the Democratic Republic of Timor-Leste), high-risk countries were prioritized for elimination of maternal and neonatal tetanus (WHO, 2002). As of 2005, 49 countries remain high risk, with expectations that this will be reduced to 35 by 2007 (Source: WHO/UNICEF MNT collected data, 2006).

The global MNT Elimination Initiative promotes a multi-faceted approach, incorporating immunization of women of childbearing age with tetanus toxoid, training of birth attendants, clean delivery practices, and improved cord care, with the aim of providing optimal protection against neonatal tetanus. In the early 1990s, when the programme had just begun, immunization efforts focused on providing pregnant women with at least two doses of TT. Subsequently, immunization was extended to adolescents and women of childbearing age, with the goal of providing protection throughout childbearing years (EPI, 1999; WHO, 2002). Data have confirmed the impact on the incidence of neonatal tetanus through immunizing all women of childbearing years (Hamid et al. 1985; Rahman et al. 1982; EPI, 1999; Koenig et al. 1998; EPI, 1996c).

An important strategy in MNT elimination has been the “high-risk” approach (EPI, 1999; WHO, 2002) which uses supplemental immunization campaigns for women of childbearing age, targeting districts with a reported incidence of disease of more than 1.0 neonatal tetanus case per 1000 births. Additional information, such as tetanus toxoid coverage, the level of clean delivery practice, and the effectiveness of surveillance, are also considered when identifying high-risk districts. The high-risk approach targets the current generation of women of childbearing age but it is essential to have immunization programmes in place to ensure each new generation has been provided with durable protective immunity. Improvements in other areas of healthcare are recognized as important factors in eliminating neonatal tetanus.

There are important factors to be considered for maintaining protective immunity in women throughout their childbearing years. Firstly, improvements in coverage with a primary series (at least three doses in the first year of life), must continue. Although it is generally accepted that the primary series in infancy only gives protection for approximately five years, it is vital in providing not only protection in the very young but the initial immunological stimulus that allows an anamnestic response to subsequent booster doses. Secondly, reinforcing doses of tetanus toxoid in children of school age and adolescents are critical in maintaining antibody

levels which can persist for decades. Evidence of this is supplied by serological surveys of countries with an established programme of tetanus immunization. However, there are many barriers preventing successful immunization throughout infancy, childhood, and adolescence in some regions, including cost, logistics, and rate of school attendance. A further compounding factor is that evidence of a complete vaccination history, verbal or written, is essential to ensure a pregnant woman is protected.

In 2006, WHO updated its tetanus policies and recommendations (EPI, 2006). The goals now are: 1) to eliminate MNT globally; 2) to achieve and sustain high coverage of three doses of DTP in infancy and of appropriate booster doses in order to prevent tetanus in all age groups. A total of six doses are recommended for an individual: three doses before the age of one year, a booster dose of tetanus toxoid-containing vaccine between 4–7 years, and a further dose in adolescence. The sixth dose is recommended for young adults to provide additional assurance of long-term protection. The WHO position paper (EPI, 2006) also provides the recommended immunization strategy for pregnant women (Table 7). The exact schedule should be flexible to maximize the healthcare services available in different countries. These recommendations aim to fulfill the goals of tetanus control, to achieve the elimination of MNT globally, and to provide long-term protection for all age groups.

**Table 7. Guidelines for tetanus toxoid (TT) immunization to obtain long term protection against tetanus**

Recommended Schedule	DTP	DTP	DTP	Td	Td	Td
	Before age one or as early as possible after age 6 weeks, with $\geq 4$ week intervals				e.g. 4–7 years	e.g. 12–15 years
Adolescents and adults with no previous immunization	Td	Td	Td	Td	Td	
	As early as possible	At least 4 weeks later	At least 6 months later	At least 1 year later	At least 1 year later	
Pregnant women with no previous immunization (or unreliable immunization)	Td	Td	Td	Td	Td	
	As early as possible in first pregnancy	At least 4 weeks later	At least 6 months later, or in next pregnancy	At least 1 year later, or in next pregnancy	At least 1 year later, or in next pregnancy	
Pregnant women with 3 childhood DTP doses	Td	Td	Td			
	As early as possible in first pregnancy	At least 4 weeks later	At least 1 year later, or in next pregnancy			
Pregnant women with 4 childhood DTP doses	Td	Td				
	As early as possible in first pregnancy	At least 1 year later, or in next pregnancy				
Supplementary immunization activities in high risk areas (women of childbearing age)	Td	Td	Td	Td	Td	
	During round 1	During round 2, at least 4 weeks after round 1	During round 3, at least 6 months after round 2	At least 1 year later, (in next pregnancy)	At least 1 year later, (in next pregnancy)	

Source: EPI, 2006

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# Annex 1:

## References

About S et al. (2000). Levels and avidity of antibodies to tetanus toxoid in children aged 1-15 years in Dar es Salaam and Bagamoyo, Tanzania. *Annals of Tropical Paediatrics*, 20:313–322.

Abrahamian et al. (2000). Fatal tetanus in a drug abuser with protective anti-tetanus antibodies. *The Journal of Emergency Medicine*, 18:189–193.

ACIP (Immunization Practices Advisory Committee, USA) (1991). Diphtheria, tetanus and pertussis: guidelines for vaccine prophylaxis and other preventative measures. Recommendations of the Immunization Practices Advisory Committee *Morbidity and mortality weekly report. CDC surveillance summaries, US Centers for Disease Control and Prevention*, 40:1–28.

ACIP (Immunization Practices Advisory Committee, USA) (1991). Update on Adult Immunization Recommendations of the Immunization Practices Advisory Committee. *Morbidity and mortality weekly report. CDC surveillance summaries, US Centers for Disease Control*, 40:1–52.

ACIP (Immunization Practices Advisory Committee, USA) (1996). Update: Vaccine side-effects, adverse reactions, contraindications and precautions. *Morbidity and mortality weekly report. CDC surveillance summaries, US Centers for Disease Control*, 45:1–35.

Aggerbeck H, Norgaard-Pedersen B, Heron I (1996). Simultaneous quantitation of diphtheria and tetanus antibodies by double antigen, time-resolved fluorescence immunoassay. *Journal of Immunological Methods*, 190:171–183.

Amstey MS et al. (1985). Fetal-neonatal passive immunization against *Hemophilus influenzae*, type b. *American Journal of Obstetrics and Gynecology*, 153:607–11.

Anderson EL, Belshe RB, Bartram J (1988). Differences in reactogenicity and antigenicity of acellular and standard pertussis vaccines combined with diphtheria and tetanus in infants. *Journal of Infectious Diseases*, 157:731–737.

Arnold RA, Soewarso TI, Karyadi A (1986). Mortality from neonatal tetanus in Indonesia: results of two surveys. *Bulletin*, Geneva, World Health Organization, 64:259–262.

Baker CJ, et al. (1988). Immunization of pregnant women with a polysaccharide vaccine of group B *Streptococcus*. *The New England Journal of Medicine*, 319: 1180–1185.

Baptist EC (1984). Tetanus in a partially immunized infant with burns. *The Pediatric Infectious Disease Journal*, 3:487–488.

- 
- Barbi M et al. (1992). Immunization in children with HIV seropositivity at birth: antibody response to polio vaccine and tetanus toxoid. *AIDS*, 6:1465–1469.
- Barington T et al. (1993). Non-epitope-specific suppression of the antibody response to *Haemophilus influenzae* type b conjugate vaccines by pre-immunization with vaccine components. *Infection and Immunity*, 61:432–438.
- Barkin RM, Samuelson JS, Gotlin LP (1984). DTP reactions and serologic response with reduced dose schedule. *The Journal of Pediatrics*, 105:189–194.
- Begg NT et al. (1995). Antibody responses and symptoms after DTP and either tetanus or diphtheria *Haemophilus influenzae* type B conjugate vaccines given for primary immunization by separate or mixed injection. *Vaccine*, 13:1547–1550.
- Beghi E et al. (1985). Brachial plexus neuropathy in the population of Rochester, Minnesota 1970–1981. *Annals of Neurology*, 18:320–323.
- Berger SA et al. (1978). Tetanus despite pre-existing anti-tetanus antibody. *JAMA: the Journal of the American Medical Association*, 240:769–770.
- Berggren GG et al. (1983). Traditional midwives, tetanus immunization and infant mortality in rural Haiti. *Tropical Doctor*, 13:79–87.
- Bildhaiya GS (1983). A study of tetanus in a hospital at Ahmedabad. *Journal of the Indian Medical Association*, 80:21–23.
- Black RE, Huber DH, Curlin GT (1980). Reduction of neonatal tetanus by mass immunization of non-pregnant women: duration of protection provided by one or two doses of aluminium-adsorbed tetanus toxoid. *Bulletin*, Geneva, World Health Organization, 58:927–930.
- Blanche S et al. (1986). Longitudinal study of 18 children with perinatal LAV/HTLV infection: attempt at prognostic evaluation. *The Journal of Pediatrics*, 109:965–970.
- Bohlke K et al. (2003). Risk of anaphylaxis after vaccination of children and adolescents. *Pediatrics*, 112: 815–820.
- Bonetti TCS et al. (2004). Tetanus and diphtheria antibodies and response to a booster dose in Brazilian HIV-1 infected women. *Vaccine*, 22:3707–3712.
- Borkowsky W et al. (1987). Antibody responses to bacterial toxoids in children infected with human immunodeficiency virus. *The Journal of Pediatrics*, 110:563–566.
- Borkowsky W et al. (1992). Cell-mediated and humoral immune responses in children infected with human immunodeficiency virus during the first four years of life. *The Journal of Pediatrics*, 120:371–375.
- Borrow R, Miller E (2006). Surrogates of protection. In: Frosch M, Maiden M, eds. *Handbook of meningococcal disease*. Switzerland, Wiley, 323–351.
- Boyd JSK (1946). Tetanus in African and European theaters of war, 1939–1945. *Lancet*, 1:113–119.
- Boyer J et al. (1953). Le tetanos en milieu urbain: conditions d'apparition-deductiona prophylactiques [Tetanus in urban areas; conditions under which it appears and preventive measures.]. *La Presse Médicale*, 61:701–703.

---

Brabin BJ et al. (1984). The influence of malaria and gestation on the immune response to one and two doses of adsorbed tetanus toxoid in pregnancy. *Bulletin*, Geneva, World Health Organization, 62:919–930.

Brair ME et al. (1994). Reduced transfer of tetanus antibodies with placental malaria. *Lancet*, 343:208–209.

Breman JG et al. (1981). The primary serological response to a single dose of adsorbed tetanus toxoid, high concentration type. *Bulletin*, Geneva, World Health Organization, 59:745–752.

Burrage M et al. (2002). Effect of vaccination with carrier protein on response to meningococcal C conjugate vaccines and value of different immunoassays as predictors of protection. *Infection and Immunity*, 70:4946–4954.

Caglar K, Karakus R, Aybay C. Determination of tetanus antibodies by a double-antigen enzyme-linked immunosorbent assay in individuals of various age groups. *European Journal of Clinical Microbiology and Infectious Diseases: official publication of the European Society of Clinical Microbiology*, 24:523–528.

Carlsson et al. (1994). Antibodies against *Haemophilus influenzae* type b and tetanus in infants after subcutaneous vaccination with PRP-T/diphtheria or PRP-OMP/diphtheria-tetanus vaccines. *The Pediatric Infectious Disease Journal*, 13:27–33.

Chatterjee SC (1964). A comparative study of the haemagglutination and bioassay procedures for the assay of guinea pig anti-diphtheria and anti-tetanus sera. *The Indian Journal of Medical Research*, 52:1241–1249.

Christensen NA, Thurber DL (1957). Clinical experience with tetanus: 91 cases. *Mayo Clinic Proceedings. Mayo Clinic*, 32:146–158.

Cliff J (1985a). Neonatal tetanus in Maputo, Mozambique. I. Hospital incidence and childbirth practices. *The Central African Journal of Medicine*, 31:9–12.

Cliff J (1985b). Neonatal tetanus in Maputo, Mozambique. II. Preventive measures. *The Central African Journal of Medicine*, 31:27–32.

Cowles PB (1937). Tetanus immunization. *The Yale Journal of Biology and Medicine*, 9:409–416.

Cox JC et al. (1983). A comparison of enzyme immunoassay and bioassay for the quantitative determination of antibodies to tetanus toxin. *Journal of Biological Standardization*, 11:123–128.

Crone NE, Reder AT (1992). Severe tetanus in immunized patients with high anti-tetanus titers. *Neurology*, 42:761–764.

Danilova E et al. (2005). Attenuated immune response to tetanus toxoid in young healthy men protected against tetanus. *Vaccine*, 23: 4980–4983.

Dagan R et al. (2004). Reduction of antibody response to an 11-valent pneumococcal vaccine coadministered with a vaccine containing acellular pertussis components. *Infection and Immunity*, 72:583–5391.

- 
- Dastur F, Awatramani V, Dixit JA (1981). Response to single dose of tetanus vaccine in subjects with naturally acquired tetanus antitoxin. *Lancet*, 2:219–221.
- Deivanayagam N, Nedunchelian K, Kamala KG (1991). Neonatal tetanus: observations on antenatal immunization, natal and immediate post-natal factors. *Indian Journal of Pediatrics*, 58:119–122.
- De Melker HE et al. (2000). A population-based study on tetanus antitoxin levels in the Netherlands. *Vaccine*, 18:100–108.
- Deming MS et al. (2002). Tetanus toxoid coverage as an indicator of serological protection against neonatal tetanus. *Bulletin*, Geneva, World Health Organization, 80:696–703.
- De Moraes-Pinto MI et al. (1995). Neonatal tetanus despite immunization and protective antitoxin antibody. *The Journal of Infectious Diseases*, 171:1076–1077.
- De Moraes-Pinto MI et al. (1996). Placental transfer and maternally acquired neonatal IgG immunity in human immunodeficiency virus infection. *The Journal of Infectious Diseases*, 173:1077–1084.
- De Moraes-Pinto MI et al. (1998). Placental antibody transfer: influence of maternal HIV infection and placental malaria. *Archives of disease in childhood. Fetal and neonatal edition*, 79:F202–F205.
- Dhillon H, Menon PS (1975). Active immunization of women in pregnancy with two injections of adsorbed tetanus toxoid for prevention of tetanus neonatorum in Punjab, India. *The Indian Journal of Medical Research*, 63:583–589.
- Dietz V et al. (1997). Factors affecting the immunogenicity and potency of tetanus toxoid: implications for the elimination of neonatal and non-neonatal tetanus as public health problems. *Bulletin*, Geneva, World Health Organization, 75(1):81–93.
- Dieye TN et al. (2002). Immunologic and virologic response after tetanus toxoid booster among HIV-1 and HIV-2-infected Senegalese individuals. *Vaccine*, 20:905–913.
- Dokmetjian J et al. (2000). A possible explanation for the discrepancy between ELISA and neutralizing antibodies to tetanus toxin. *Vaccine*, 18:2698–2703.
- Edsall G et al. (1967). Excessive use of tetanus toxoid boosters. *JAMA: the Journal of the American Medical Association*, 202:17–19.
- Edsall G et al. (1975). Malnutrition and the immune response to tetanus toxoid. In: *Proceedings of the fourth international conference on tetanus, Dakar, 6–12 April 1975*. Lyon, Fondation Merieux, 683–685.
- Edsall G (1976). Problems in the immunology and control of tetanus. *The Medical Journal of Australia*, 2:216–220.
- Edwards KM et al. (1989). Evaluation of a new highly purified pertussis vaccine in infants and children. *The Journal of Infectious Diseases*, 160:832–837.
- Einterz EM, Bates ME (1991). Caring for neonatal tetanus patients in a rural primary care setting in Nigeria: a review of 237 cases. *Journal of Tropical Pediatrics*, 37:179–181.

- 
- Eisen AH, Cohen JJ, Rose B (1963). Reaction to tetanus toxoid: a report of a case with immunologic studies. *The New England Journal of Medicine*, 269:1408–1411.
- El-Sherbini A (1991). Study of tetanus neonatorum in Tanta Fever Hospital, 1988-1989. *Journal of Tropical Pediatrics*, 37:262–263.
- Ergonul O, Sozen T, Tekeli E (2001). Immunity to tetanus among adults in Turkey. *Scandinavian Journal of Infectious Diseases*, 33:728–730.
- Evans DG (1943). Persistence of antitoxin in man following active immunization. *Lancet*, 2:316–317.
- Gad ZM et al (1986). An epidemiological study on tetanus neonatorum in Alexandria. *The Bulletin of the High Institute of Public Health*, 16:33–45.
- Galazka AM. *The Immunological Basis for Immunization Series Module 3: Tetanus*. Geneva, World Health Organization, 1993 (WHO/EPI/Gen 93.13). [http://www.who.int/vaccines-documents/DocsPDF-IBI-e/mod3\\_e.pdf](http://www.who.int/vaccines-documents/DocsPDF-IBI-e/mod3_e.pdf).
- Galazka A, Milstien J, Zaffran M (1998). *Thermostability of vaccines*. Geneva, World Health Organization. <http://www.who.int/vaccines-documents/DocsPDF/www9661.pdf>.
- Gendrel D et al. (1990a). Transfert placentaire des anticorps antitetaniques et protection du nouveau-ne [Placental transfer of tetanus antibodies and protection of newborn infants]. *Archives françaises de pédiatrie*, 47:725–729.
- Gendrel D et al. (1990b). Placental transfer of tetanus antibodies and protection of the newborn. *Journal of Tropical Pediatrics*, 36:279–282.
- Ghosh JB (1990). Prevention of tetanus neonatorum. *Indian Pediatrics*, 27:210.
- Gidding HF et al. (2005). Immunity to diphtheria and tetanus in Australia: a national serosurvey. *The Medical Journal of Australia*, 183:301–304.
- Gilles HM et al. (1983). The Malumphasi Project — an epidemiological, clinical and laboratory study. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 77:24–31.
- Gold H (1937). Studies on tetanus toxoid. I. Active immunization of allergic individuals with tetanus toxoid, alum precipitated, refined. *The Journal of Allergy*, 8:230–245.
- Goulon M et al. (1972). Les anticorps antitetaniques. Titrage avant seroanatoxiotherapie chez 64 tetaniques [Antitetanus antibodies. Assay before anatoxiotherapy in 64 tetanus patients]. *La Nouvelle Presse Médicale*, 1:3049–3050.
- Grange AO (1991). Neonatal tetanus in Lagos Metropolis. *Nigerian Journal of Paediatrics*, 18:12–21.
- Greenwood BM et al. (1972). Immunosuppression in children with malaria. *Lancet*, 1:169–172.
- Grudeborn B (1987). 199 casos de tetano neonatal em Luanda [199 cases of neonatal tetanus in Luanda]. *Acta Media Angola*, 6:17–33.

---

Gupta RK, Griffin P Jr, Rivera R, Siber GR (1998). Development of an animal model to assess the immunogenicity of single-dose tetanus and diphtheria vaccines based on controlled release from biodegradable polymer microspheres. *Developments in Biological Standardization*, 92:277–287.

Gupta RK, Maheshwari SC, Singh H (1984). The titration of tetanus antitoxin. III. A comparative evaluation of indirect haemagglutination and toxin neutralization titres of human sera. *Journal of Biological Standardization*, 12:145–149.

Gupta RK, Maheshwari SC, Singh H (1985). The titration of tetanus antitoxin. IV. Studies on the sensitivity and reproducibility of the toxin neutralization test. *Journal of Biological Standardization*, 13:143–149.

Gupta RK, Siber GR (1994). Comparative analysis of tetanus antitoxin titers of sera from immunized mice and guinea pigs determined by toxin neutralization test and enzyme-linked immunosorbent assay. *Biologicals: Journal of the International Association of Biological Standardization*, 22:215–219.

Hagenaars AM, Van Delft RW, Nagel J (1984). Comparison of ELISA and toxin neutralization for the determination of tetanus antibodies. *Journal of Immunoassay*, 5:1–11.

Hall WW (1948). US Navy's war record with tetanus toxoid. *Annals of Internal Medicine*, 28:275–277.

Hamati-Haddad A, Fenichel GM (1997). Brachial neuritis following routine childhood immunization for diphtheria, tetanus and pertussis (DTP): Report of two cases and review of literature. *Pediatrics*, 99:602–603.

Hamid ED et al. (1985). Tetanus neonatorum in babies delivered by traditional birth attendants in Medan, Indonesia. *Paediatrica Indonesiana*, 25:167–174.

Hardegree MC et al. (1970). Immunization against neonatal tetanus in New Guinea. 2. Duration of primary antitoxin responses to adjuvant tetanus toxoids and comparison of booster responses to adjuvant and plain toxoids. *Bulletin*, Geneva, World Health Organization, 43:439–451.

Hedrick EC (1953). Tetanus: 2 cases in immunized persons. *California Medicine*, 79:49–50.

Hendriksen CFM et al. (1988). The toxin binding inhibition test as a reliable in vitro alternative to the toxin neutralization test in mice for the estimation of tetanus antitoxin in human sera. *Journal of Biological Standardization*, 16: 287–297.

Hendriksen C, Winsnes R (2002). Serological methods for potency testing of tetanus toxoid vaccines for human use. *Developments in Biologicals*, 111:131–40.

Hlady WG et al. (1992). Neonatal tetanus in rural Bangladesh: Risk factors and toxoid efficacy. *American Journal of Public Health*, 82: 1365–1369.

Holliday PL, Bauer RB (1983). Polyradiculoneuritis secondary to immunization with tetanus and diphtheria toxoids. *Archives of Neurology*, 40:56–57.

Holmes SJ et al. (1993). Immunogenicity of *Haemophilus influenzae* type b polysaccharide-tetanus toxoid conjugate vaccine in infants. *American Journal of Diseases of Children* (1911), 147:832–836.

---

Huang LM et al. (2005). Immunogenicity and reactogenicity of a reduced-antigen-content diphtheria-tetanus-acellular pertussis vaccine in healthy Taiwanese children and adolescents. *The Journal of Adolescent Health: official publication of the Society for Adolescent Medicine*, 37:517.e1-517.e5.

Institute of Medicine (1994). Vaccine Safety Committee. Diphtheria and tetanus toxoids. Adverse events associated with childhood vaccines: evidence bearing on causality. In: Stratton KR, Howe CJ, Johnston RB, eds. *Research strategies for assessing adverse effects associated with vaccines*. Washington, DC, National Academy Press, 67–117.

Jaganathan KS et al. (2005). Development of a single dose tetanus toxoid formulation based on polymeric microspheres: a comparative study of poly (D,L-lactic-co-glycolic acid) versus chitosan microspheres. *International Journal of Pharmaceutics*, 294:23–32.

Jodar L et al. (2003a). Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. *Vaccine*, 21:3265–3272.

Jodar L et al. (2003b). Meningococcal conjugate vaccine for Africa: a model for development of new vaccines for the poorest countries. *Lancet*, 361:1902–1904.

Johansen P et al. (2000). Towards clinical testing of a single-administration tetanus vaccine based on PLA/PLGA microspheres. *Vaccine*, 19:1047–1054.

Kipper MJ et al. (2006). Single dose vaccine based on biodegradable polyanhydride microspheres can modulate immune response mechanism. *Journal of Biomedical Materials Research. Part A*, 76:798–810.

Kitchin NRE et al. (2007). Evaluation of a diphtheria-tetanus-acellular-pertussis-inactivated poliovirus-Hib vaccine given concurrently with meningococcal group C conjugate vaccine at 2, 3 and 4 months of age. *Archives of Disease in Childhood*, 92:11–16.

Knutsson N et al. (2001). Immunogenicity and reactogenicity of diphtheria, tetanus and pertussis toxoids combined with inactivated polio vaccine, when administered concomitantly with or as a diluent for a Hib conjugate vaccine. *Vaccine*, 19 :4396–4403.

Koenig MA et al. (1998). Duration of protective immunity conferred by maternal tetanus toxoid immunization: further evidence from Matlab, Bangladesh. *American Journal of Public Health*, 88:903–907.

Kristiansen M, Aggerbeck H, Heron I (1997). Improved ELISA for determination of anti-diphtheria and/or anti-tetanus antitoxin antibodies in sera. *APMIS: acta pathologica, microbiologica, et immunologica Scandinavica*, 105:843–853.

Kroon FP et al. (1999). Immunoglobulin (IgG) subclass distribution and IgG1 avidity of antibodies in human immunodeficiency virus-infected individuals after revaccination with tetanus toxoid. *Clinical and Diagnostic Laboratory Immunology*, 6:352–355.

---

Kryl R, Prasilova F, Neubertova E (1964). Prevention du tetanos ombilical et puerperal par la vaccination pratiquée chez les femmes enceintes et influence de l'immunité passive sur l'immunité active de leurs enfants [Prevention of umbilical and puerperal tetanus through vaccination done on pregnant women and influence of passive immunity on active immunity of their infants]. *La Clinica Pediatrica (Bologna)*, 46:509–517.

Kumar V et al. (1988). Neonatal tetanus mortality in a rural community of Haryana. *Indian Pediatrics*, 25:167–169.

Kurrika S et al. (1996). Passively acquired anti-tetanus and anti-*Haemophilus* antibodies and the response to *Haemophilus influenzae* type b-tetanus toxoid conjugate vaccine in infancy. *The Pediatric Infectious Disease Journal*, 15:530–535.

Kurtzhals JAL et al. (1992). Immunity against diphtheria and tetanus in human immunodeficiency virus –infected Danish men born 1950–1959. *APMIS: acta pathologica, microbiologica, et immunologica Scandinavica*, 100:803–808.

Kurtzhals JAL et al. (1997). Immunity to tetanus and diphtheria in rural Africa. *The American Journal of Tropical Medicine and Hygiene*, :56:576–579.

Lahiri DC (1939). Absence of specific antitoxin in persons exposed to risk of tetanus infection. *The Indian Journal of Medical Research*, 27:581–583.

Levine L, Wyman L (1964). A nationwide serum survey of United States military recruits, 1962. V. Serologic immunity to tetanus. *American Journal of Hygiene*, 80:314–319.

Levine L et al. (1966). Active-passive tetanus immunization. *The New England Journal of Medicine*, 274:186–190.

Levine L, Edsall G (1981). Tetanus toxoid: what determines reaction proneness? *The Journal of Infectious Diseases*, 144:376.

Lin TY et al. (2003). Safety and immunogenicity of a diphtheria, tetanus, and acellular pertussis-inactivated poliovirus vaccine/*Haemophilus influenzae* type B combination vaccine administered to Taiwanese infants at 2, 4, and 6 months of age. *Chang Gung Medical Journal*, 26:315–22.

Lloyd JC et al. (2003). Adverse event reporting rates following tetanus-diphtheria and tetanus toxoid vaccinations: data from the Vaccine Adverse Event Reporting System (VAERS) 1991–1997. *Vaccine*, 21:3746–3750.

Lodha R et al. (2000). Tetanus in immunized children. *Indian Pediatrics*, 37:223–224.

Long AP (1954). Immunization to tetanus. *Industrial Medicine and Surgery*, 23:275–277.

Looney JM et al. (1956). Persistence of antitoxin levels after tetanus toxoid inoculation in adults, and effect of a booster dose after various intervals. *The New England Journal of Medicine*, 254:6–12.

Macko MB (1985). Comparison of the morbidity of tetanus toxoid boosters with tetanus-diphtheria toxoid boosters. *Annals of Emergency Medicine*, 14:33–35.

- 
- MacLennan R (1981). Prevention of neonatal tetanus in developing countries. In: *Proceedings of the sixth international conference on tetanus, Lyon, France, 3–5 December 1981*. Lyon, Fondation Merieux:113–121.
- MacLennan R et al. (1965). Immunization against neonatal tetanus in New Guinea: antitoxin response of pregnant women to adjuvant and plain toxoids. *Bulletin*, Geneva, World Health Organization, 32:683–697.
- Mallet E et al. (2004). A liquid hexavalent combined vaccine against diphtheria, tetanus, pertussis, poliomyelitis, *Haemophilus influenzae* type B and hepatitis B: review of immunogenicity and safety. *Vaccine*, 22: 1343–1357.
- Maple CPA et al. (2001). Immunity to diphtheria and tetanus in England and Wales. *Vaccine*, 19:167–173.
- Maru M, Getahun A, Hosana S (1988). A house-to-house survey of neonatal tetanus in urban and rural areas in the Gondar region, Ethiopia. *Tropical and Geographical Medicine*, 40:233–236.
- Maselle SY et al. (1991). Neonatal tetanus despite protective serum antitoxin concentration. *FEMS Microbiology Immunology*, 3:171–175.
- Mathur GP et al. (1980). Tetanus neonatorum — its epidemiology and management. *Indian Pediatrics*, 17:797–801.
- Matzkin H, Regev S (1985). Naturally acquired immunity to tetanus toxin in an isolated community. *Infection and Immunity*, 48:267–268.
- McCarroll J, Abrahams I, Skudder PA (1962). Antibody response to tetanus toxoid 15 years after initial immunization. *American Journal of Public Health*, 52:1669–1675.
- McCormick JB et al. (1980). Antibody response to serogroup A and C meningococcal polysaccharide vaccines in infants born of mothers vaccinated during pregnancy. *The Journal of Clinical Investigation*, 65:1141–1144.
- McQuillan GM et al. (2002). Serologic immunity to diphtheria and tetanus in the United States. *Annals of Internal Medicine*, 136:660–666.
- Melville-Smith ME, Seagroatt VA, Watkins JT (1983). A comparison of enzyme-linked immunosorbent assay (ELISA) with the toxin neutralization test in mice as a method for estimation of tetanus antitoxin in human sera. *Journal of Biological Standardization*, 11:137–144.
- Melvin AJ, Mohan KM (2003). Response to immunization with measles, tetanus, and *Haemophilus influenzae* type b vaccines in children who have human immunodeficiency virus type 1 infection and are treated with highly active antiretroviral therapy. *Pediatrics*, 111:641–644.
- Misra SR, Rao RS (1988). Serological survey of tetanus antibodies in the Pondicherry region. *The Indian Journal of Medical Research*, 87:161–165.

---

Monjour L et al. (1982). Étude de l'immunité humorale et cellulaire après vaccination antitetanique chez l'enfant africain malnutrié et paludéen. 1. Étude de la réponse en anticorps tetaniques. [Humoral and cellular immunity following antitetanus vaccination in malnourished and malaria-induced African children. 1. Study of the antitetanus antibody response] *Bulletin*, Geneva, World Health Organization, 60:589–596.

Moss GWO, Waters GG, Brown MH (1955). Efficacy of tetanus toxoid. *Canadian Journal of Public Health. Revue Canadienne de Santé Publique*, 46:142–147.

Moss WJ et al. (2003). Immunization of children at risk of infection with human immunodeficiency virus. *Bulletin*, Geneva, World Health Organization, 81:6170.

Myers et al. (1982). Primary immunization with tetanus and diphtheria toxoids: reaction rate and immunogenicity in older children and adults. *JAMA: the Journal of the American Medical Association*, 248:2478–2480.

Newell KW et al. (1966). The use of tetanus toxoid for the prevention of neonatal tetanus in developing countries for the prevention of tetanus neonatorum. *Bulletin*, World Health Organization, 35:863–871.

Newell KW et al. (1971). The serological assessment of a tetanus toxoid field trial. *Bulletin*, World Health Organization, 45:773–785.

Newton N, Janati A (1987). Guillain-Barré syndrome after vaccination with purified tetanus toxoid. *The Southern Medical Journal*, 80:1053–1054.

Nohynek H et al. (1999). Effect of transplacentally acquired tetanus antibodies on the antibody responses to *Haemophilus influenzae* type b-tetanus toxoid conjugate and tetanus toxoid vaccines in Filipino infants. *The Pediatric Infectious Disease Journal*, 18:25–30.

Okoko BJ et al. (2001). Influence of placental malaria infection and maternal hypergammaglobulinaemia on matron-foetal transfer of measles and tetanus antibodies in a rural West African population. *Journal of Health, Population and Nutrition*, 19:59–65.

Ourth DD, MacDonald AB (1977). Neutralization of tetanus toxin by human and rabbit immunoglobulin classes and subunits. *Immunology*, 33:807–815.

Owa JA, Makinde OO (1990). Neonatal tetanus in babies of immunized mothers. *Journal of Tropical Pediatrics*, 36:143–144.

Oyedeyji GA, Olamijulo SK, Joiner KT (1982). Neonatal tetanus in Ilesa, Nigeria: a review of the present status. *Nigerian Medical Journal: journal of the Nigeria Medical Association*, 12:131–135.

Pascual FB et al. (2003). Tetanus surveillance — United States, 1998–2000. Morbidity and mortality weekly report. CDC surveillance summaries, US Centers for Disease Control and Prevention, 52:1–8.

Passen EL, Andersen BR (1986). Clinical tetanus despite a “protective” level of toxin-neutralizing antibody. *JAMA: the Journal of the American Medical Association*, 255:1171–1173.

- 
- Peel MM (1980). Measurement of tetanus antitoxin. II. Toxin neutralization. *Journal of Biological Standardization*, 8:191–207.
- Peterson H-I (1965). A case of tetanus in spite of active toxoid prophylaxis. *Acta Chirurgica Scandinavica*, 129:235–237.
- Peyre M et al. (2003). An experimental divalent vaccine based on biodegradable microspheres induces protective immunity against tetanus and diphtheria. *Journal of Pharmaceutical Sciences*, 92:957–966.
- Peyre M et al. (2004). Childhood and malaria vaccines combined in biodegradable microspheres produce immunity with synergistic interactions. *Journal of Controlled Release. Official journal of the Controlled Release Society*, 99:345–355.
- Pichichero ME, Barkin RM, Samuelson JS (1986). Pediatric diphtheria and tetanus toxoids-adsorbed vaccine: immune response to the first booster following the diphtheria and tetanus toxoid vaccine primary series. *The Pediatric Infectious Disease Journal*, 5:428–430.
- Pickering JW et al. (2002). Comparison of a multiplex flow cytometric assay with enzyme-linked immunosorbent assay for quantitation of antibodies to tetanus, diphtheria and *Haemophilus influenzae* type b. *Clinical and Diagnostic Laboratory Immunology*, 9:872–876.
- Pollard JD, Selby G (1978). Relapsing neuropathy due to tetanus toxoid: report of a case. *Journal of the Neurological Sciences*, 37:113–125.
- Pryor T, Onarecker C, Coniglione T (1997). Elevated abtotoxin titers in a man with generalized tetanus. *The Journal of Family Practice*, 44:299–303.
- Quast U, Hennesen W, Widmark RM (1979). Mono- and polyneuritis after tetanus vaccination (1970–1977). *Developments in Biological Standardization*, 43:25–32.
- Rahman M et al. (1982). Use of tetanus toxoid for the prevention of neonatal tetanus. 1. Reduction of neonatal mortality by immunization of non-pregnant and pregnant women in rural Bangladesh. *Bulletin*, Geneva, World Health Organization, 60:261–267.
- Ramon G (1936). L'anatoxine tetanique et la prophylaxie du tetanos chez l'homme et chez les animaux domestiques [Tetanus antitoxin and the prevention of tetanus in man and domestic animals]. *La Presse Médicale*, 44:1625–1628.
- Ramsay ME et al. (1993). Antibody response to accelerated immunization with diphtheria, tetanus, pertussis vaccine. *Lancet*, 342:203–5.
- Rantala J et al. (1994). Epidemiology of Guillain-Barré syndrome in children: relationship of oral polio vaccine occurrence. *The Journal of Pediatrics*, 124:220–223.
- Ratliff DA, Burns-Cox CJ (1983). Anaphylaxis to tetanus toxoid (unreviewed reports). *British Medical Journal*, (Clinical Research Edition), 288:114.
- Ray SN et al. (1978). Sero-survey of diphtheria and tetanus antitoxin. *The Indian Journal of Medical Research*, 68:901–904.

---

Redwan ERM, Al-Awady MK (2002). Prevalence of tetanus immunity in the Egyptian population. *Human Antibodies*, 11:55–59.

Relihan M (1969). Reactions to tetanus toxoid. *Journal of the Irish Medical Association*, 62: 430–434.

Rey M (1981). Discussion. In: *Proceedings of the sixth international conference on tetanus, Lyon, France, 3–5 December 1981*. Lyon, Fondation Merieux, 178.

Richmond P et al. (2001). Evaluation of De-O-acetylated meningococcal C polysaccharide-tetanus toxoid conjugate vaccine in infancy: reactogenicity, immunogenicity, immunologic priming, and bactericidal activity against O-acetylated and De-O-acetylated serogroup C strains. *Infection and Immunity*, 69:2378–2382.

Rosen JB, Breman JG (2004). Malaria intermittent preventative treatment in infants, chemoprophylaxis, and childhood vaccinations. *Lancet*, 1386–1388.

Rosenblatt HM et al. (2005). Tetanus immunity after diphtheria, tetanus toxoids and acellular pertussis vaccination in children with clinically stable HIV infection. *The Journal of Allergy and Clinical Immunology*, 116:698–703.

Ryder RW et al. (1993). Safety and immunogenicity of bacille Calmette-Guérin, diphtheria-tetanus-pertussis, and oral polio vaccines in newborn children in Zaire infected with human immunodeficiency virus type 1. *The Journal of Pediatrics*, 122:697–702.

Sachs A (1952). Modern views on prevention of tetanus in wounded. *Proceedings of the Royal Society of Medicine*, 45:641–652.

Sadeghi-Hasanabadi A (1987). Neonatal tetanus in Fars Province, Islamic Republic of Iran. *Eastern Mediterranean Region Health Service Journal*, 3:14–21.

Saenger R et al. (2005). Booster vaccination with hexavalent DTPa-HBV-IPV/Hib vaccine in the second year of life is as safe as concomitant DTPa-IPV/Hib + HBV administered separately. *Vaccine*, 23:1135–1143.

Sangpetchsong V et al. (1985). Effect of passive immunity to tetanus in DTP vaccinated infants. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 16:117–123.

Scheifele DW, Guasparini R, Lavigne P (1999). A comparative study of PENTA vaccine booster doses given at 12, 15, or 18 months of age. *Vaccine*, 17:543–50.

Scott S et al. (2005). Neonatal measles immunity in rural Kenya: the influence of HIV and placental malaria infections on placental transfer of antibodies and levels of antibody in maternal and cord serum samples. *The Journal of Infectious Diseases*, 191:1854–1860.

Sedgwick AK et al. (1983). Rapid quantitative microenzyme-linked immunosorbent assay for tetanus antibodies. *Journal of Clinical Microbiology*, 18:104–109.

Sesardic D et al. (1993). The first international standard for anti-tetanus immunoglobulin, human: pharmaceutical evaluation and international collaborative study. *Biologicals. Journal of the International Association of Biological Standardization*, 21:67–75.

- 
- Shumacker HB Jr, Lamont A (1942). Some problems concerning active immunization against tetanus. *Surgery, Gynecology and Obstetrics*, 74:433–438.
- Siber GR (1997). Methods for estimating serological correlates of protection. *Developments in Biologicals (Basel)*, 89:283–296.
- Simonsen O et al. (1984). Immunity against tetanus and revaccination 25–30 years after primary vaccination. *Lancet*, 2: 1240–1242.
- Simonsen O et al. (1987a). Evaluation of vaccination requirements to secure continuous antitoxin immunity to tetanus. *Vaccine*, 5:115–121.
- Simonsen O et al. (1987c). Revaccination against tetanus 17 to 20 years after primary vaccination: Kinetics of antibody response. *The Journal of Trauma*, 27:1358–1361.
- Simonsen O, Bentzon MW, Heron I (1986). ELISA for the routine determination of antitoxic immunity to tetanus. *Journal of Biological Standardization*, 14:231–239.
- Simonsen O, Schou C, Heron I (1987b). Modification of the ELISA for the estimation of tetanus antitoxin in human sera. *Journal of Biological Standardization*, 15: 143–157.
- Sisk CW, Lewis CE (1965). Reactions to tetanus-diphtheria toxoid (adult). *Archives of Environmental Health*, 11:34–36.
- Sneath PAT, Kerslake EG (1935). Persistence of tetanus antitoxin in man following active immunization. *British Medical Journal*, 2:290.
- Sneath PAT, Kerslake EG, Sruby F (1937). Tetanus immunity: resistance of guinea pigs to lethal spore doses induced by active and passive immunization. *American Journal of Hygiene*, 25:464–476.
- Spittle BJ, Pollock M, O'Donnell TV (1973). Tetanus occurring in spite of active immunization. *The New Zealand Medical Journal*, 77:250.
- Stanfield JP, Gall D, Bracken PN (1973). Single dose antenatal tetanus immunization. *Lancet*, 1:215–219.
- Stark K et al. (1999). Seroprevalence and determinants of diphtheria, tetanus and poliomyelitis among adults in Berlin, Germany. *Vaccine*, 17:844–850.
- Stiffler-Rosenberg G, Fey H (1975). Radioimmunologische Messung von Tetanusantitoxin [Radioimmunological measurement of tetanus antitoxin]. *Schweizerische Medizinische Wochenschrift*, 105:804–810.
- Stroffolini T et al. (1997). Immunity to tetanus in the 3–20 year age group in Italy. *Public Health*, 111:19–21.
- Stroh G et al. (1987). Measurement of mortality from neonatal tetanus in Burma. *Bulletin World Health Organization*, 65:309–316.
- Swartz TA et al. (2003). Immune response to a diphtheria and tetanus toxoid administration in a three-dose diphtheria tetanus whole-cell pertussis/enhanced inactivated poliovirus vaccination schedule: a 7-year follow-up. *European Journal of Epidemiology*, 18:827–33.

---

Symeonidis N et al. (2003). Serological survey of immunity to tetanus in adult population of Northern Halkidiki, Greece. *European Journal of Epidemiology*, 18:1147–1152.

Talesnik E et al. (1998). Time course of antibody response to tetanus toxoid and pneumococcal capsular polysaccharides in patients infected with HIV. *Journal of acquired immune deficiency syndromes and human retrovirology: official publication of the International Retrovirology Association*, 19:471–476.

Tasman A, Huygen FJA (1962). Immunization against tetanus of patients given injections of anti-tetanus serum. *Bulletin*, Geneva, World Health Organization, 397–407.

Tenbroeck C, Bauer JH (1923). Studies on the relation of tetanus bacilli in the digestive tract to tetanus antitoxin in the blood. *The Journal of Experimental Medicine*, 37:479–489.

Trinca JC (1974). Antibody response to successive booster doses of tetanus toxoid in adults. *Infection and Immunity*, 10:1–5.

Tsairis P, Dyck PJ, Mulder DW (1965). Natural history of brachial plexus neuropathy: report on 99 patients. *Archives of Neurology*, 2:116–120.

Turner TB et al. (1954). Studies on the duration of protection afforded by active immunization against tetanus. *Bulletin of the Johns Hopkins Hospital*, 86:205–217.

Tuttle J et al. (1997). The risk of Guillain-Barré syndrome after tetanus-toxoid-containing vaccines in adults and children in the United States. *American Journal of Public Health*, 87:2045–8.

United Kingdom Department of Health Immunization against infectious diseases, (2006). “*The Green Book*”. Chapter 30 Tetanus. <http://www.dh.gov.uk/assetRoot/04/14/13/52/04141352.pdf>. [Last accessed 01/03/2007].

Vandelaer J et al. (2003). Tetanus in developing countries: an update on the maternal and neonatal tetanus elimination initiative. *Vaccine*, 21: 3442–3445.

Vergara R et al. (2005). Reduced-antigen-content-diphtheria-tetanus-acellular-pertussis and inactivated polio vaccine as a booster for adolescents 10 to 14 years of age. *European Journal of Pediatrics*, 164:377–82.

Verma M, Dhanwade SM, Singh T (1989). Clinical study of tetanus neonatorum. *Indian Journal of Medical Sciences*, 43:235–238.

Veronesi R et al. (1975). Naturally acquired tetanus immunity: further evidence in human and animals. In: *Proceedings of the fourth international conference on tetanus, Dakar, Senegal, 6–12 April 1975*. Lyon, Fondation Merieux, 613–626.

Veronesi R et al. (1981). Naturally acquired tetanus immunity: further evidence in humans and animals from the Galapagos Islands. In: *Proceedings of the sixth international conference on tetanus, Lyon, France, 3–5 December 1981*. Lyon, Fondation Merieux, 243–249.

Virella G, Hyman B (1991). Quantitation of anti-tetanus and anti-diphtheria antibodies by enzyme immunoassay: methodology and applications. *Journal of Clinical Laboratory Analysis*, 5:43–48.

---

Volk VK et al. (1962). Antigenic response to booster dose of diphtheria and tetanus toxoids. *Public Health Reports*, 77:185–194.

Wassilak SGF et al. (2004). Tetanus Toxoid. In: Plotkin SA, Orenstein WA, eds. *Vaccines* 4th ed., Saunders, Philadelphia, Ch. 27:745–781.

White WG et al. (1973). Reactions to tetanus toxoid. *The Journal of Hygiene (London)*, 71:283–297.

Winsnes R, Christiansen G (1979). Quantification of tetanus antitoxin in human sera. *Acta pathologica et microbiologica Scandinavica*, 87:197–200.

Wolters KL, Dehmel H (1938). Über den verlauf der aktiven und aktiv-passiven immunität bei tetanus [On the course of active and active-passive immunity against tetanus]. *Zeitschrift J. Infektionskr*, 53:140–147.

Wolters KL, Dehmel H (1942). Abschliessende Untersuchungen über die Tetanusprophylaxe durch active Immunisierung [Final investigations on tetanus prophylaxis, through active immunization]. *Zeitschrift fuer Hygiene und Infektionskrankheiten*, 124:381–332.

World Health Organization, Expanded Programme on Immunization (1982). Prevention of neonatal tetanus. *Weekly Epidemiological Record*, 57:137–142. [http://whqlibdoc.who.int/wer/WHO\\_WER\\_1982/WER1982\\_57\\_137-144%20\(N°18\).pdf](http://whqlibdoc.who.int/wer/WHO_WER_1982/WER1982_57_137-144%20(N°18).pdf) [Last accessed 30/11/2006].

World Health Organization, Expanded Programme on Immunization (1987). Neonatal tetanus mortality surveys, Egypt. *Weekly Epidemiological Record*, 62:332–335. [http://whqlibdoc.who.int/wer/WHO\\_WER\\_1987WER1987\\_62\\_329-336%20\(N°44\).pdf](http://whqlibdoc.who.int/wer/WHO_WER_1987WER1987_62_329-336%20(N°44).pdf) [Last accessed 30/11/2006].

World Health Organization, Expanded Programme on Immunization (1996a): Estimating tetanus protection of women by serosurvey. *Weekly Epidemiological Record*, 71:117–124. <http://www.who.int/docstore/wer/pdf/1996/wer7116.pdf> [Last accessed 30/11/2006].

World Health Organization, Expanded Programme on Immunization (1996b): The children's vaccine initiative and the global programme for vaccines and immunization. Part I. *Weekly Epidemiological Record*, 71:261–268. <http://www.who.int/docstore/wer/pdf/1996/wer7135.pdf> [Last accessed 30/11/2006]

World Health Organization, Expanded Programme on Immunization (1996c). Progress towards neonatal tetanus elimination, 1988-1994. *Weekly Epidemiological Record*, 71:33–36. <http://www.who.int/docstore/wer/pdf/1996/wer7105.pdf> [Last accessed 30/11/2006].

World Health Organization, Expanded Programme on Immunization (1999): Progress towards the global elimination of neonatal tetanus, 1990–1998. *Weekly Epidemiological Record*, 74:73–80. <http://www.who.int/docstore/wer/pdf/1999/wer7410.pdf> [Last accessed 30/11/2006].

World Health Organization, Expanded Programme on Immunization (2001): Assessment of elimination of neonatal tetanus, Zimbabwe. *Weekly Epidemiological Record*, 2001, 76:101–106. <http://www.who.int/docstore/wer/pdf/2001/wer7614.pdf> [Last accessed 30/11/2006].

---

World Health Organization, Expanded Programme on Immunization (2002): Assessment of neonatal tetanus elimination, Namibia. *Weekly Epidemiological Record*, 77:121–123. <http://www.who.int/docstore/wer/pdf/2002/wer7715.pdf> [Last accessed 30/11/2006].

World Health Organization, Expanded Programme on Immunization (2006): Tetanus vaccine. *Weekly Epidemiological Record*, 81:198–208. <http://www.who.int/wer/2006/wer8120.pdf> [Last accessed 30/11/2006].

World Health Organization (1999) Field manual for neonatal tetanus elimination. Geneva, World Health Organization, (WHO/V&B/99.14) <http://www.who.int/vaccines-documents/DocsPDF/www9563.pdf>. [Last accessed 30/11/2006].

World Health Organization, (2002) United Nations Children's Fund (UNICEF) and United Nations Population Fund (UNFPA). Maternal and Neonatal Tetanus Elimination by 2005: Strategies for Achieving and Maintaining Elimination, November 2000. *World Health Organization (WHO/V&B/02.09)*. <http://www.who.int/vaccines-documents/DocsPDF02/www692.pdf> [Last accessed 30/11/2006].

Yuan L et al. (1997). Diphtheria and tetanus immunity among blood donors in Toronto. *Canadian Medical Association Journal*, 156:985–990.

Zalogna GP, Chernow B (1982). Life-threatening anaphylactic reactions to tetanus toxoid. *Annals of Allergy*, 48:107–108.

Zuger B, Greenwald CK, Gerber H (1942). Tetanus immunization: effectiveness of stimulating dose of toxoid under conditions of infection. *Journal of Immunology (Baltimore, Md.: 1950)*, 44:309–317.





The World Health Organization has managed cooperation with its Member States and provided technical support in the field of vaccine-preventable diseases since 1975. In 2003, the office carrying out this function was renamed the WHO Department of Immunization, Vaccines and Biologicals.

The Department's goal is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases. Work towards this goal can be visualized as occurring along a continuum. The range of activities spans from research, development and evaluation of vaccines to implementation and evaluation of immunization programmes in countries.

WHO facilitates and coordinates research and development on new vaccines and immunization-related technologies for viral, bacterial and parasitic diseases. Existing life-saving vaccines are further improved and new vaccines targeted at public health crises, such as HIV/AIDS and SARS, are discovered and tested (*Initiative for Vaccine Research*).

The quality and safety of vaccines and other biological medicines is ensured through the development and establishment of global norms and standards (*Quality Assurance and Safety of Biologicals*).

The evaluation of the impact of vaccine-preventable diseases informs decisions to introduce new vaccines. Optimal strategies and activities for reducing morbidity and mortality through the use of vaccines are implemented (*Vaccine Assessment and Monitoring*).

Efforts are directed towards reducing financial and technical barriers to the introduction of new and established vaccines and immunization-related technologies (*Access to Technologies*).

Under the guidance of its Member States, WHO, in conjunction with outside world experts, develops and promotes policies and strategies to maximize the use and delivery of vaccines of public health importance. Countries are supported so that they acquire the technical and managerial skills, competence and infrastructure needed to achieve disease control and/or elimination and eradication objectives (*Expanded Programme on Immunization*).

## **Department of Immunization, Vaccines and Biologicals**

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