The immunological basis for immunization series

Module 3: Tetanus

Update 2006

The Department of Immunization, Vaccines and Biologicals
thanks the donors whose unspecified financial support
has made the production of this document possible.

This module is a revision of Artur Galazka's 1st edition and was produced for Immunization, Vaccines and Biologicals, WHO, by:

- Dr Ray Borrow PhD, Vaccine Evaluation Unit, Health Protection Agency, Manchester Royal Infirmary, Manchester, UK;
- Dr Paul Balmer PhD, Vaccine Evaluation Unit, Health Protection Agency, Manchester Royal Infirmary, Manchester, UK;
- Dr Martha H. Roper, MD, MPH, Independent Consultant, Middlebury, USA.

Printed in February 2007

Copies of this publications as well additional materials
on immunization, vaccines and biological may be requested from:
World Health Organization
Department of Immunization, Vaccines and Biologicals
CH-1211 Geneva 27, Switzerland
• *Fax:* + 41 22 791 4227 • *Email:* vaccines@who.int •

Printed by the WHO Document Production Services, Geneva, Switzerland

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	*Haemophilus influenzae* 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

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

Preface

This module is part of the Series "The Immunological Basis for Immunization", which was initially developed in 1993 as a set of eight modules focusing on the vaccines included in the Expanded Programme on Immunization (EPI)[1]. In addition to a general immunology module, each of the seven other modules covered one of the vaccines recommended as part of the EPI programme, 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*) 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*).

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.

[1] This programme was established in 1974 with the main aim of providing immunization for children in developing countries.

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 gangloside-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.

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.

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.

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.

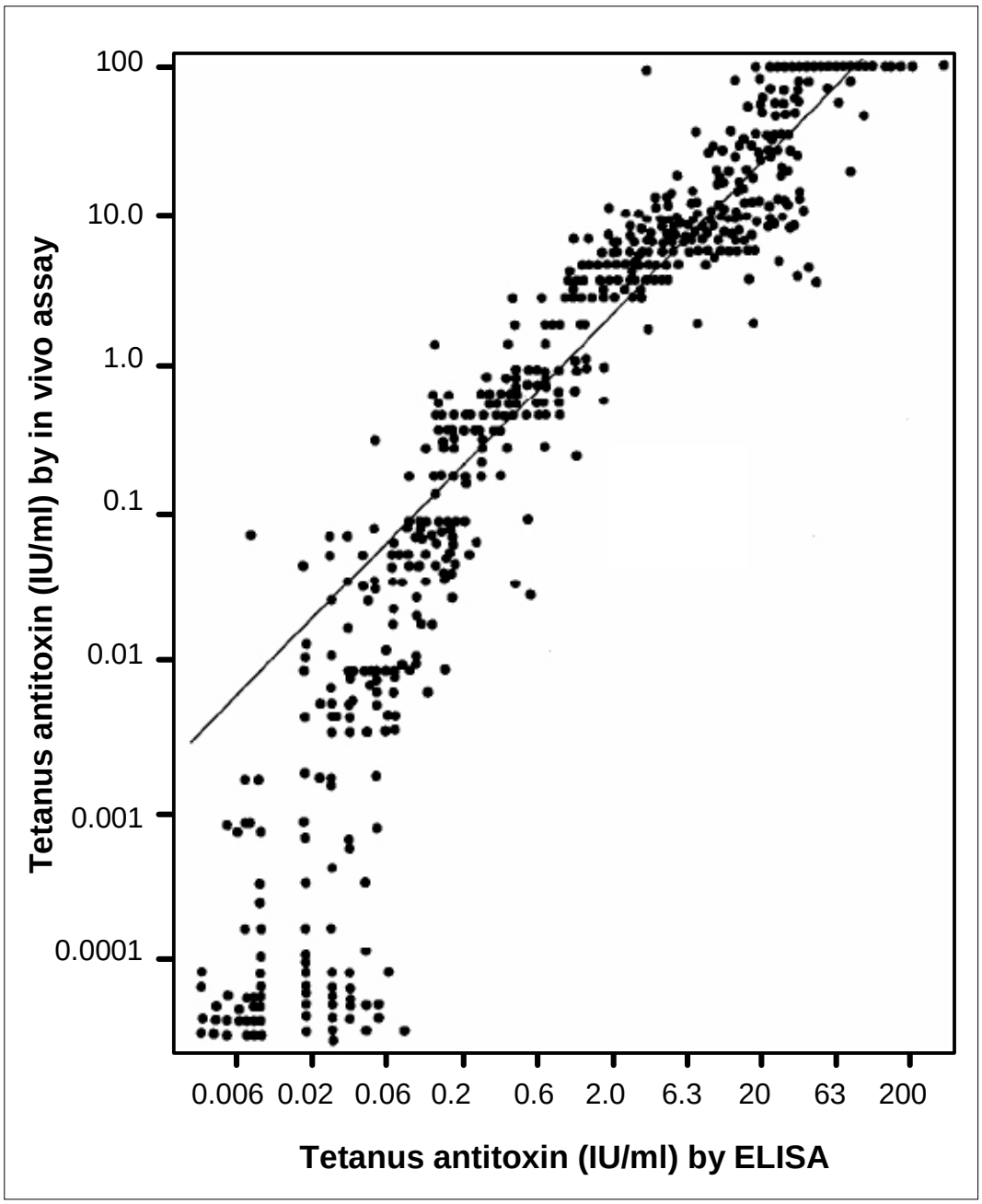
3.2.2 ELISA

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; Hagenaars 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).

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.

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 an 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 & Dehemel, 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.

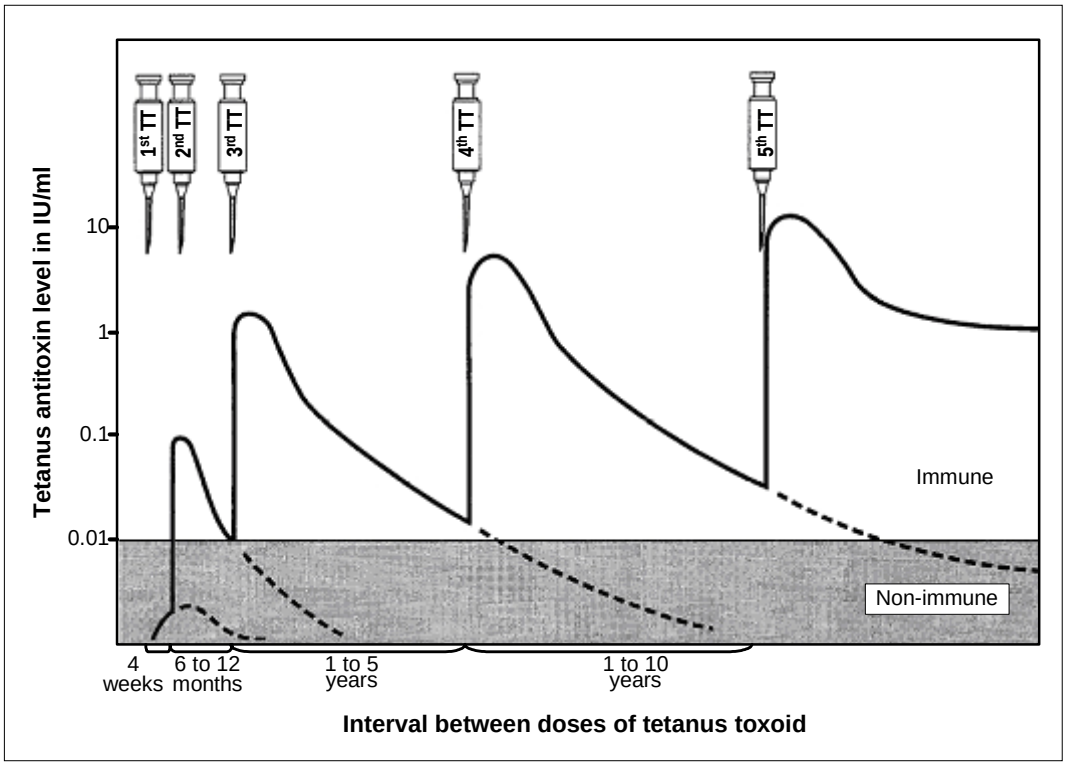
5. Development of immunity following immunization

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/m1 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.

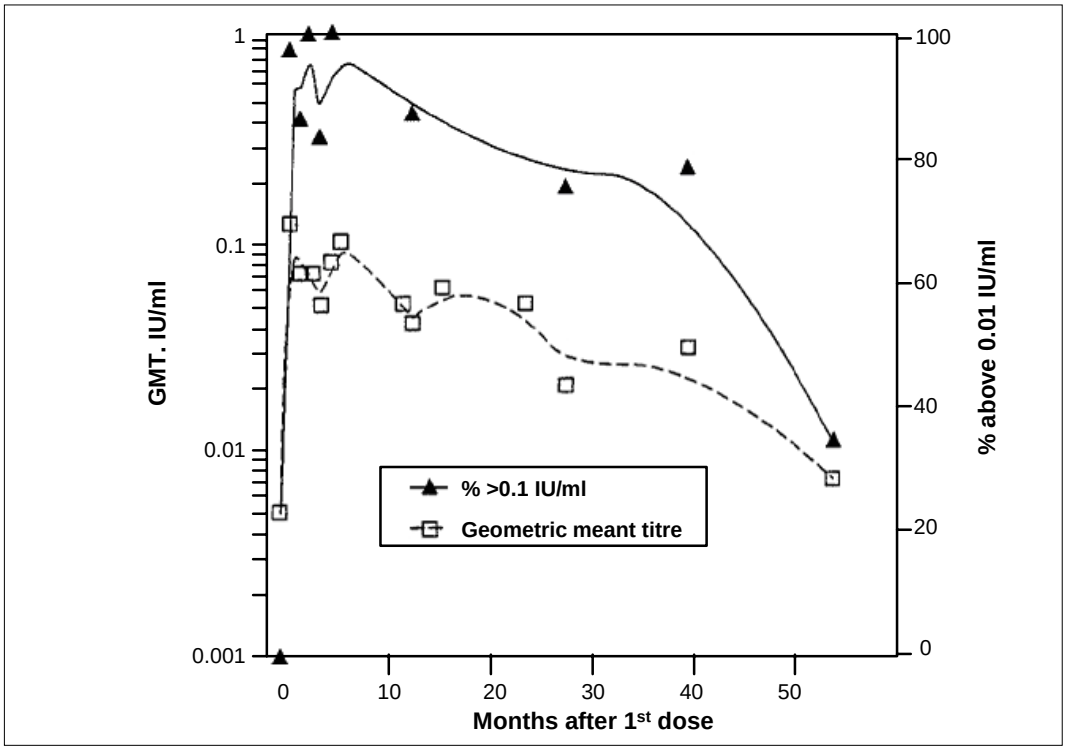
In children, three primary doses of DTP vaccine induce an antibody level above the minimum protective threshold, with a mean level above 0.2 IU/ml (Anderson et al. 1988; Barkin et al. 1984; Edwards et al. 1989; Pichichero et al. 1986). Factors influencing the height of the immune response in children 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, Hardegree et al. 1970).

5.2 Duration of immunity following various immunization schedules

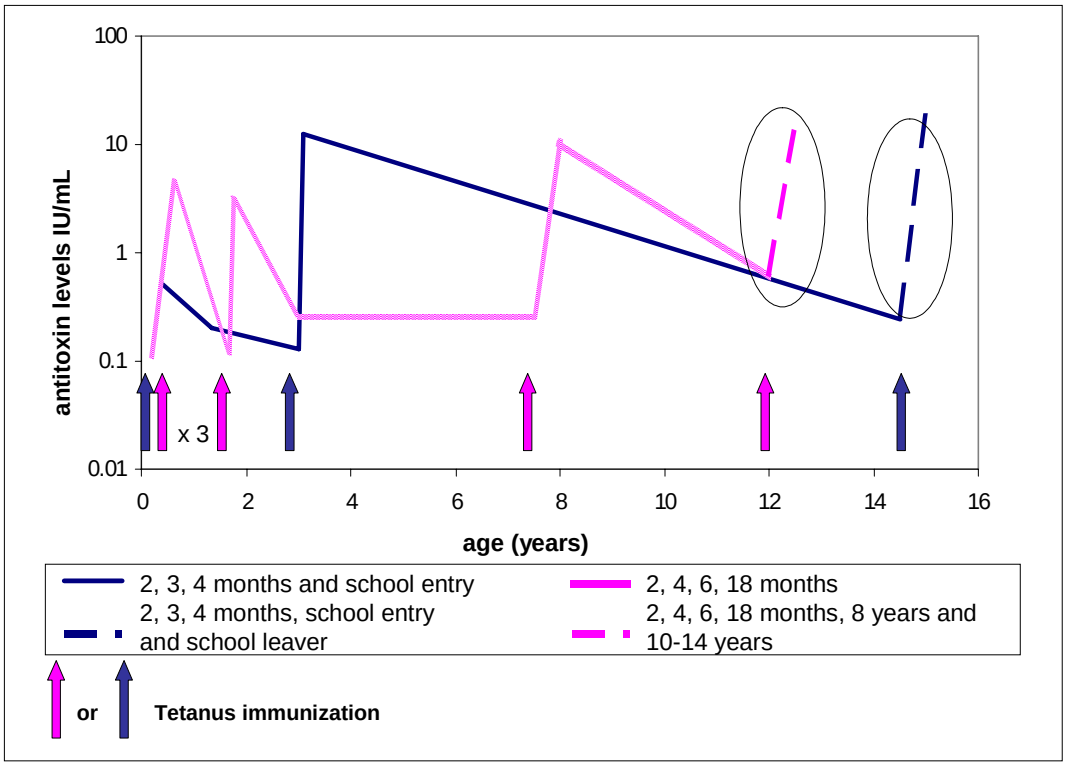
An understanding of the duration of immunity induced by immunization has important implications for the recommendations on the number and timing of doses to be given.

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 ≥ 0.1 IU/mL were observed in 97% and 54% of children aged 1–5 and 6–15 years respectively (Aboud et al. 2000).

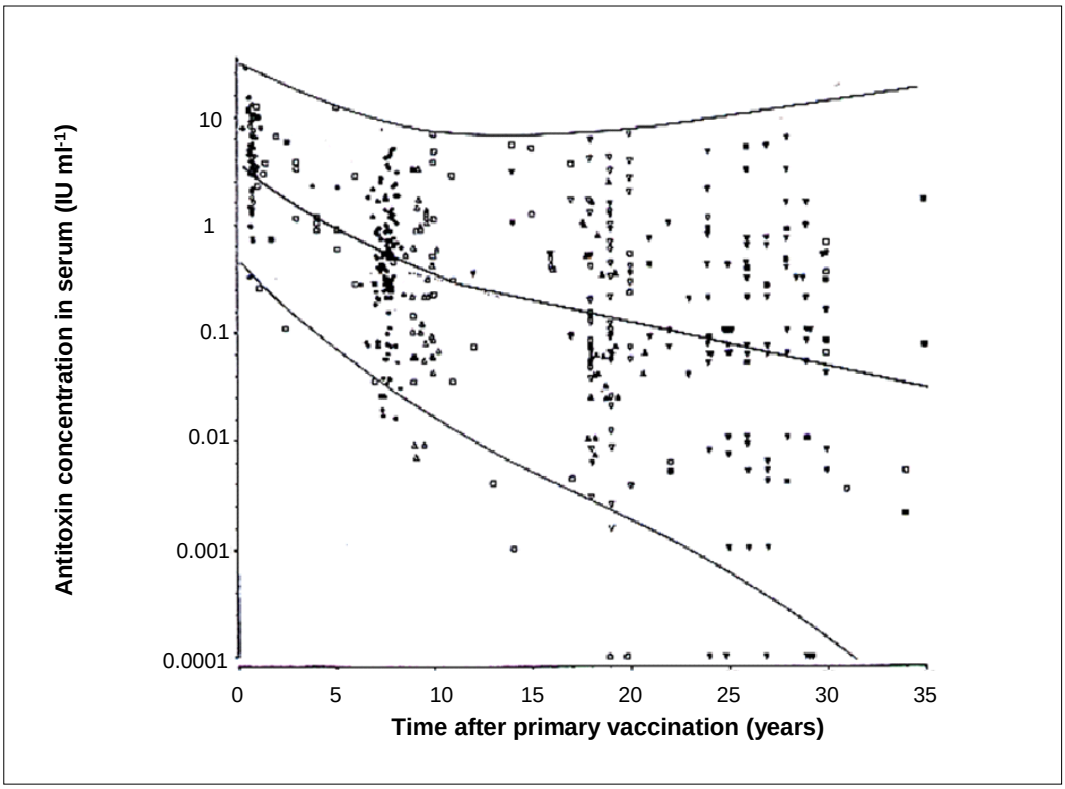
Figure 4. Response to tetanus immunization following various schedules



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).

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.

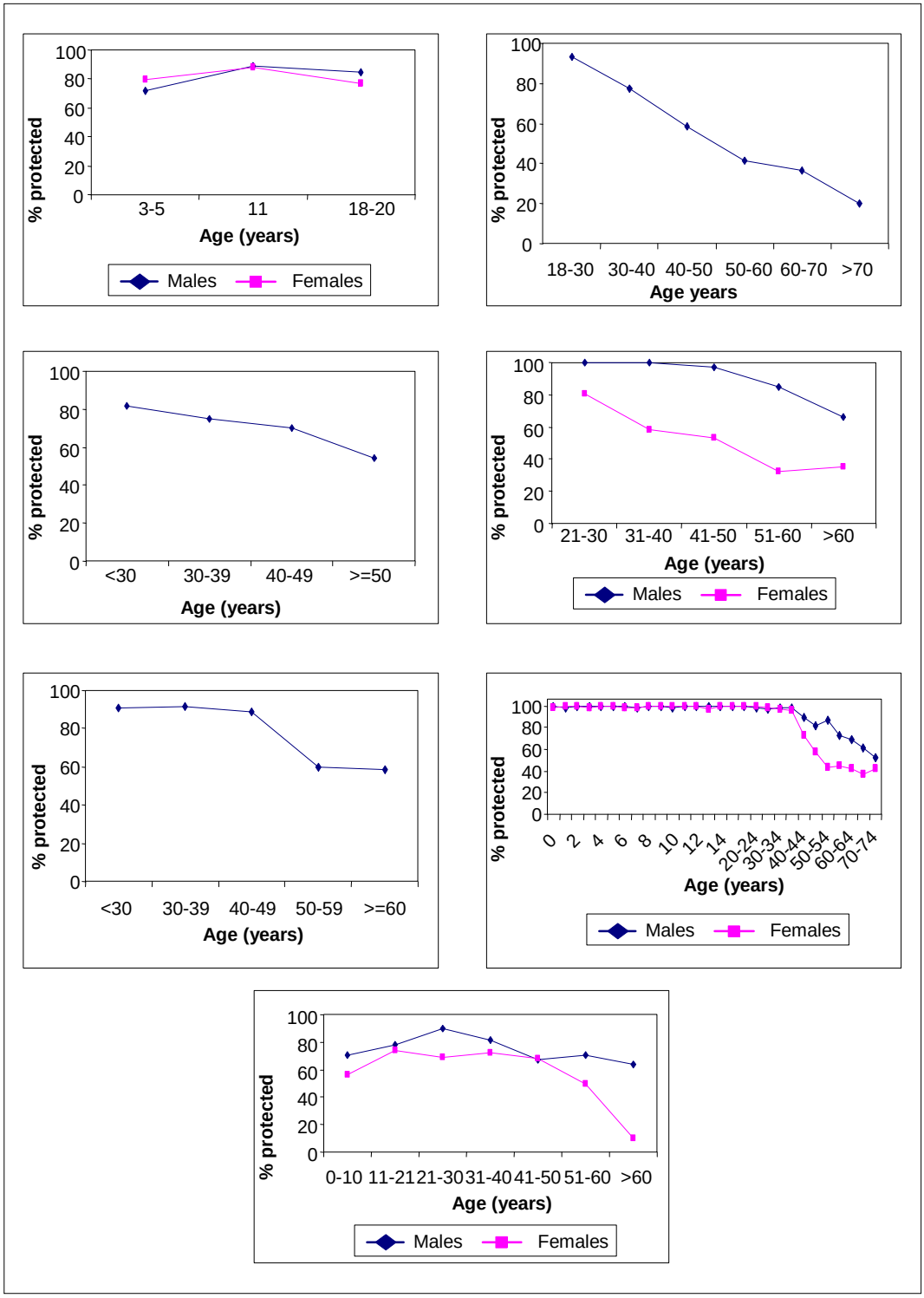
The kinetics of the response to a tetanus booster is of importance, both to predict protection of neonates after administration of boosters to pregnant women, and because it is recommended as a part of management of patients with tetanus-prone wounds. The median period of incubation to onset of tetanus 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

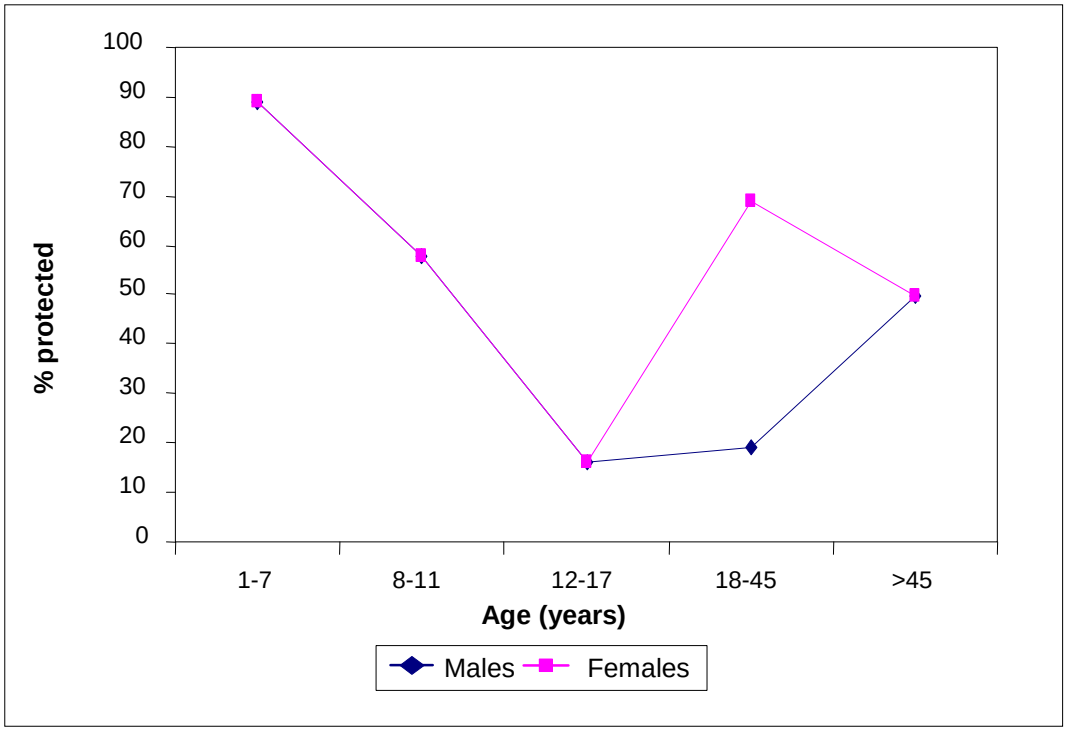


	Country	Reference	Assay	Protective Threshold (IU/mL)	Schedule
a)	Italy	Stroffolini et al. 1997	PHA	0.1	3, 5, 11 months, 5–6 years (+ every 10 years)
b)	Turkey	Ergonul et al. 2001	ELISA	0.01	2, 3, 4, 18 months, 6 & 13 years
c)	Germany	Stark et al. 1999	ELISA	0.1	3, 4, 5, 12–18 months, 3 & 8 years
d)	Greece	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.

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.

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.

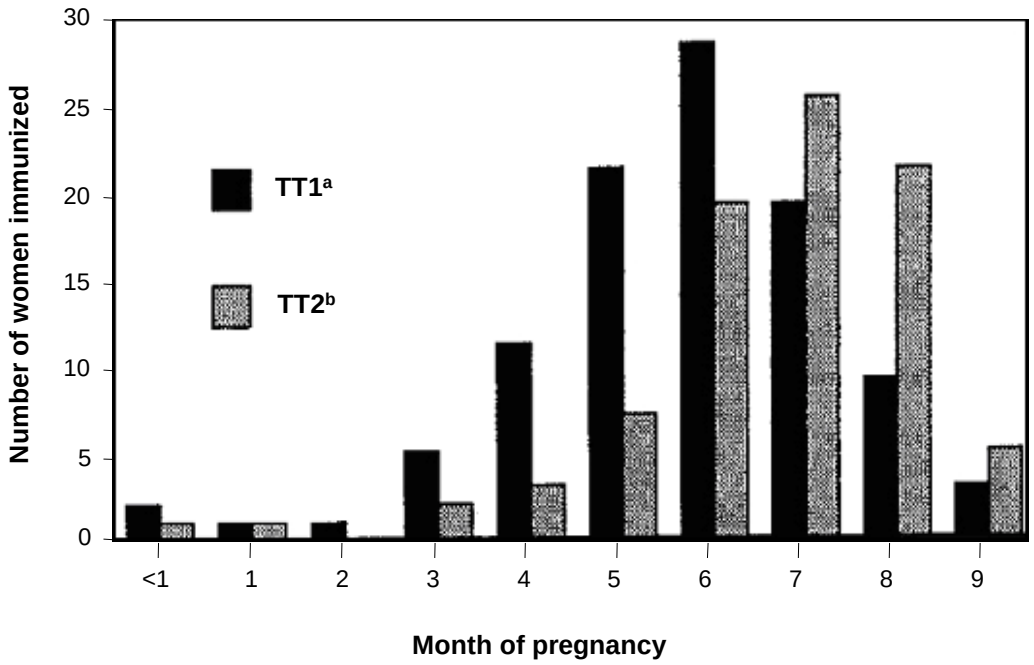
Nonetheless, even if women first present to health services late in pregnancy, the opportunity should be taken to administer primary (or booster) immunization(s) if indicated, in order to contribute to long-lasting immunity, and protection in subsequent pregnancies.

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

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



[a] Mean time for TT1 = 5.7 months
[b] Mean time for TT2 = 6.6 months

Source: Galzaka, 1993.

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 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).

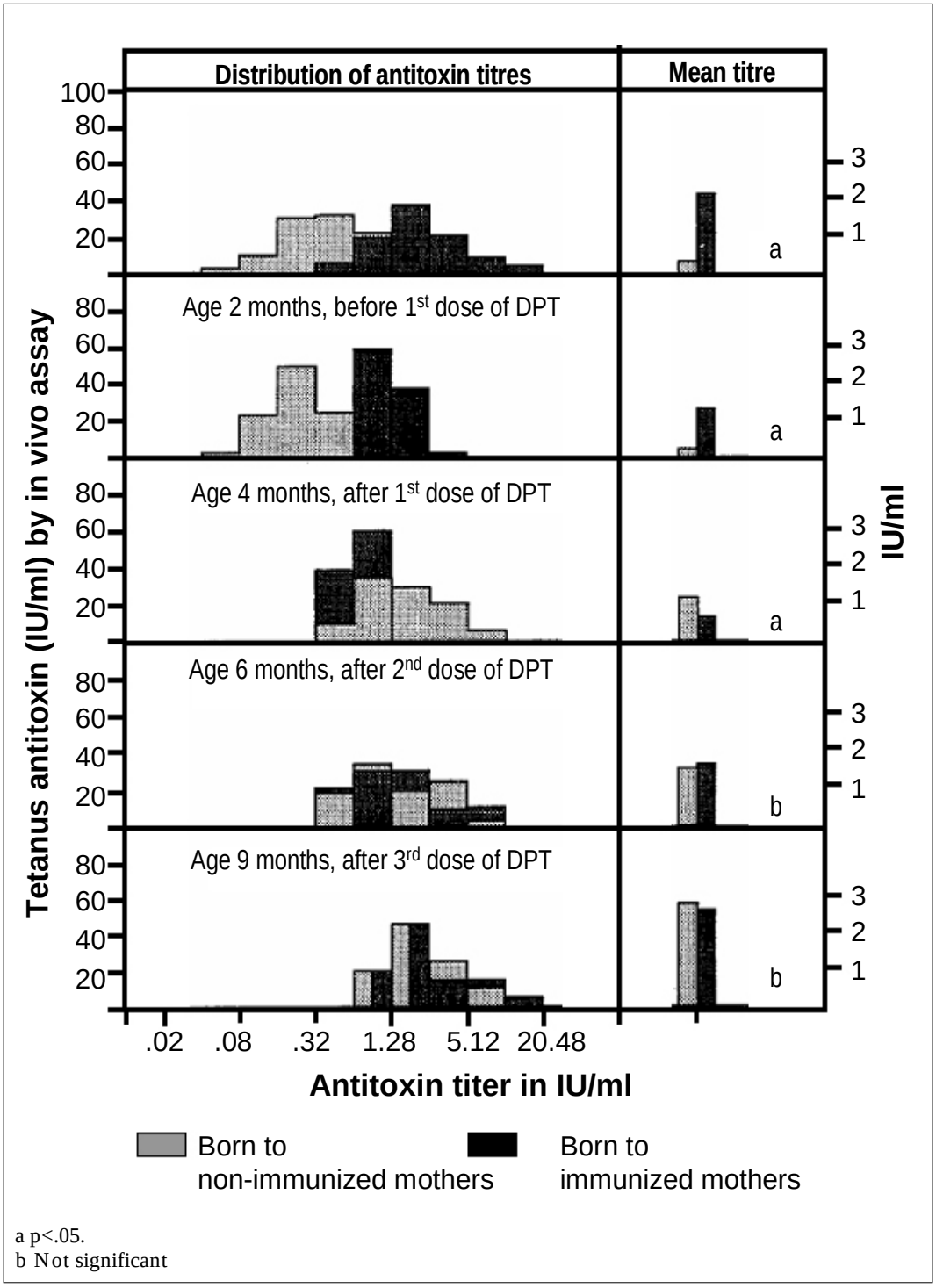
6.4 Interference between passive antibodies and development of active immunity

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



a $p<.05$.
b Not significant

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