WHO Immunological Basis for Immunization Series

Module 3: Tetanus
Update 2018

Immunization, Vaccines and Biologicals

World Health Organization

The immunological basis for immunization series: module 3: tetanus (Immunological basis for immunization series ; module 3)

ISBN 978-92-4-151361-6

Suggested citation. The immunological basis for immunization series: module 3: tetanus. Geneva: World Health Organization; 2018 (Immunological basis for immunization series; module 3). Licence: *CC BY-NC-SA 3.0 IGO*.

Cataloguing-in-Publication (CIP) data. CIP data are available at *http://apps.who.int/iris*.

Sales, rights and licensing. To purchase WHO publications, see *http://apps.who.int/bookorders*. To submit requests for commercial use and queries on rights and licensing, see *http://www.who.int/about/licensing*.

WHO Immunological Basis for Immunization Series

Module 3: Tetanus
Update 2018

Immunization, Vaccines and Biologicals

World Health Organization

Contents

Abbreviations and acronyms

ACIP	Advisory Committee on Immunization Practices
ADEM	acute disseminated encephalomyelitis
AEFI	adverse events following immunization
AIDS	acquired immunodeficiency syndrome
CDC	Centers for Disease Control and Prevention (USA)
CIDP	chronic inflammatory disseminated polyneuropathy
Da	Dalton (mass spectrometry)
DTaP	DT-acellular pertussis vaccine
DTP	diphtheria-tetanus-pertussis vaccine
DT	diphtheria-tetanus vaccine for children
EPI	Expanded Programme on Immunization
ELISA	enzyme-linked immunosorbent assay
GBS	Guillain-Barré Syndrome
HA	passive haemagglutination (test)
Hib	Haemophilus influenzae type b
HIV	human immunodeficiency virus
Ig	immunoglobulin
IgG	immunoglobulin G
IgM	immunoglobulin M
IOM	Institute of Medicine (USA)
ITP	immune thrombocytopenic purpura
IU	international units
kg	kilogram
LF	limits of flocculation
mL	millilitre
MNTE	maternal and neonatal tetanus elimination
MNT	Maternal and Neonatal Tetanus

NA	neutralization assay
ng	nanogram
OMS	opsoclonus myoclonus syndrome
PRP	polyribosylribitol phosphate
RIA	radioimmunoassay
RSV	respiratory syncytial virus
SIA	supplementary immunization activities
SIDS	sudden infant death syndrome
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
TTCV	tetanus toxoid-containing vaccine
UNICEF	United Nations Children's Fund
VMMC	voluntary male medical circumcision
WHO	World Health Organization

Preface

This module is part of the WHO series The immunological basis for immunization, which was initially developed in 1993 as a set of eight modules, comprising one module on general immunology and seven modules each devoted to one of the vaccines recommended for the Expanded Programme on Immunization – i.e. vaccines against diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. Since then, this series has been updated and extended to include other vaccines of international importance. The main purpose of the modules is to provide national immunization managers and vaccination professionals with an overview of the scientific basis of vaccination against a range of important infectious diseases. The modules developed since 1993 continue to be vaccine-specific, reflecting the biological differences in immune responses to the individual pathogens and the differing strategies employed to create the best possible level of protection that can be provided by vaccination. The modules also serve as a record of the immunological basis for the WHO recommendations on vaccine use, as published in the WHO vaccine position papers.[1]

[1] See: *http://www.who.int/immunization/documents/positionpapers_intro/en/index.html*, accessed 31 July 2018.

Acknowledgements

The preparation of this publication was coordinated by the Director´s office of the WHO Department of Immunization, Vaccines, and Biologicals. WHO thanks the donors whose unspecified financial support has made the production of this document possible.

This module was updated for WHO by **Nicole E. Basta**, Assistant Professor, Division of Epidemiology and Community Health, University of Minnesota, USA; **Ray Borrow**, Vaccine Evaluation Unit, Public Health England, Manchester, United Kingdom and **Elizabeth Miller**, Immunisation Department, Public Health England, London, United Kingdom. No conflict of interest was declared.[2]

WHO thanks those who provided expert and technical reviews for the initial preparation of the module and the 2018 update: Thomas Cherian, WHO; Kyla Hayford, Division: Global Disease Epidemiology and Control, Johns Hopkins Bloomberg School of Public Health; Dianliang Lei, WHO; Elisabeth Raquel Krow-Lucal U.S. Centers for Disease Control and Prevention; Heather Scobie, U.S. Centers for Disease Control and Prevention.

[2] According to WHO's Guidelines for Declaration of Interests (WHO expert), an interest is considered "personal" if it generates financial or nonfinancial gain to the expert, such as consulting income or a patent. "Specificity" indicates whether the declared interest is a subject matter of the meeting or work to be undertaken. An interest has "financial significance" if the honoraria, consultancy fees or other received funding, including those received by expert's organization, from any single vaccine manufacturer or other vaccine-related company exceeds US$ 5000 in a calendar year. Likewise, a shareholding in any one vaccine manufacturer or other vaccine-related company in excess of US$ 1000 would also constitute a "significant shareholding".

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. C. tetani* is not an invasive organism; infection with *C. tetani* remains localized. Tetanus 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 contaminated with tetanus spores are applied to the umbilical stump. Non-neonatal tetanus (i.e. after 28 days of life) may occur through open wounds resulting from injuries, nosocomial infections (e.g. surgery, circumcision, abortion), or even tungiasis (i.e. chigger/jigger infestation).

Tetanus toxin is produced by *C. tetani* bacteria as a single polypeptide chain of 150 000 Da and then cleaved into two linked polypeptides – a 100 000 Da heavy chain and a 50 000 Da light chain. The toxin 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. The 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. This action of tetanus toxin is thought to result in most of the characteristic disease pathogenesis – i.e. muscle spasm and rigidity.

2. Tetanus toxoid and the nature of immunity against tetanus

2.1 Tetanus toxoid vaccine

Tetanus toxin can be inactivated by formaldehyde to make tetanus toxoid (TT). TT is available as either a single-antigen vaccine or in combination vaccines to protect against other vaccine-preventable diseases, including diphtheria, pertussis, poliomyelitis, hepatitis B and illness caused by *Haemophilus influenzae* type b (Hib). The pentavalent vaccine, which provides protection against diphtheria, tetanus, pertussis, Hib and hepatitis B (DTP-Hib-HepB), is the most commonly used childhood vaccine worldwide, but other pentavalent (DTaP-IPV/Hib) and hexavalent (DTaP-IPV/Hib-HepB) combinations are also available. For booster dosing, a tetanus-diphtheria combination with lower concentration of diphtheria antigen (d) is available. Use of tetanus-toxoid-containing vaccine (TTCV) combinations with diphtheria toxoid is strongly encouraged and single antigen vaccines should be discontinued whenever feasible in order to help maintain high immunity to both diphtheria and tetanus throughout the life course. TT is adsorbed onto aluminium salts (aluminium hydroxide or aluminium phosphate) to increase its antigenicity. The potent immunogenicity of TT has led to its use as a protein carrier in polysaccharide-protein conjugate vaccines, including Hib, meningococcal (A, C, ACWY and combinations C-Hib, CY-Hib), pneumococcal (PCV) and typhoid (TCV) conjugate vaccines (see Section 9 – *Combination vaccines and concomitant vaccine use*). TT is stable; it can also 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, Milstien & Zaffran, 1998). Vaccines containing TT must not be frozen (WHO, 2006).

Work is in progress in a number of areas to improve the delivery and immunogenicity of TT vaccines. The use of chitosan nanospheres loaded with TT which can be delivered by a mucosal route is being investigated in animal models (Pirouzmand, Khameneh & Tafaghodial, 2017; Barhate et al., 2014).

2.2 Tetanus toxoid-induced immunity

TT induces the formation of specific antibodies called 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 the 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 is enough to cause the infection but is insufficient to stimulate generation of protective antibody levels. Consequently, all patients with clinical tetanus should be immunized with TTCVs, 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, Awatramani & Dixit, 1981; Matzkin & Regev, 1985; Tenbroeck & Bauer, 1923; Veronesi, Correia & Ferreira, 1975; Veronesi et al., 1983). However, studies of tetanus antibodies among persons who are said to be unvaccinated were unable to exclude the possibility of prior, unreported vaccination (MacLennan, 1981). 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 low-resource settings, natural immunity is not thought to have any practical importance in preventing or 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 because 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 serum (Sesardic et al., 1993). An international standard serum, TE-3 WHO reference serum is available. 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 (e.g. the disease symptoms or death of mice) can influence outcome and hence antibody levels. Furthermore, the accuracy of the assay depends on the potency of the toxin and weight of the mice (Gupta, Maheshwari & Singh, 1985; Peel, 1980). It is therefore clear that, although considered to be the "gold standard" assay, there is no internationally standardized protocol available and it is very difficult to compare results directly from different studies (and antibody levels) performed by different laboratories. Because of the expensive and labour-intensive nature of the in vivo assay and the need for large numbers of animals, an internationally standardized protocol has not been 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, the radioimmunoassay, standard or modified enzyme-linked immunosorbent assays (ELISAs), and bead-based immunofluorescence assays. With the exception of the radioimmunoassay, 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 (Ourth & MacDonald, 1977). Other in vitro tests have been shown to have non-specific binding (e.g. low affinity interactions) at low antibody ranges that may be incompatible with certain assay uses. Therefore, the results of in vitro techniques should be interpreted carefully and verified where possible against the in vivo neutralization method.

3.2.1 Passive haemagglutination

The passive haemagglutination (HA) test is a simple in vitro assay where TT-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 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, Maheshwari & Singh , 1984; Gupta, Maheshwari & Singh, 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 antibodies. The HA test is used infrequently now in the determination of antitoxin levels.

3.2.2 Radioimmunoassay

Radioimmunoassay (RIA) tests have been used to titrate tetanus antibodies. There are several possible modifications of the RIA test: TT can be coupled with an insoluble sorbent, such as cellulose or agarose (Stiffler-Rosenberg & Fey, 1975), or adsorbed passively onto a plastic surface as in the ELISA test. The specific antibodies bind to the antigen immunosorbent and are quantified by measuring the incorporation of isotope-labelled human antiglobulin attached to the antigen-antibody complex. The sensitivity of the RIA test is high and the results correlate well with values obtained by the HA test (Wang et al. 1982) and the ELISA test (Layton, 1980; Stiffler-Rosenberg & Fey, 1977). However, the reagents and equipment needed for the RIA test are expensive and the technique can be used only by highly trained personnel.

3.2.3 Commonly used ELISAs

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

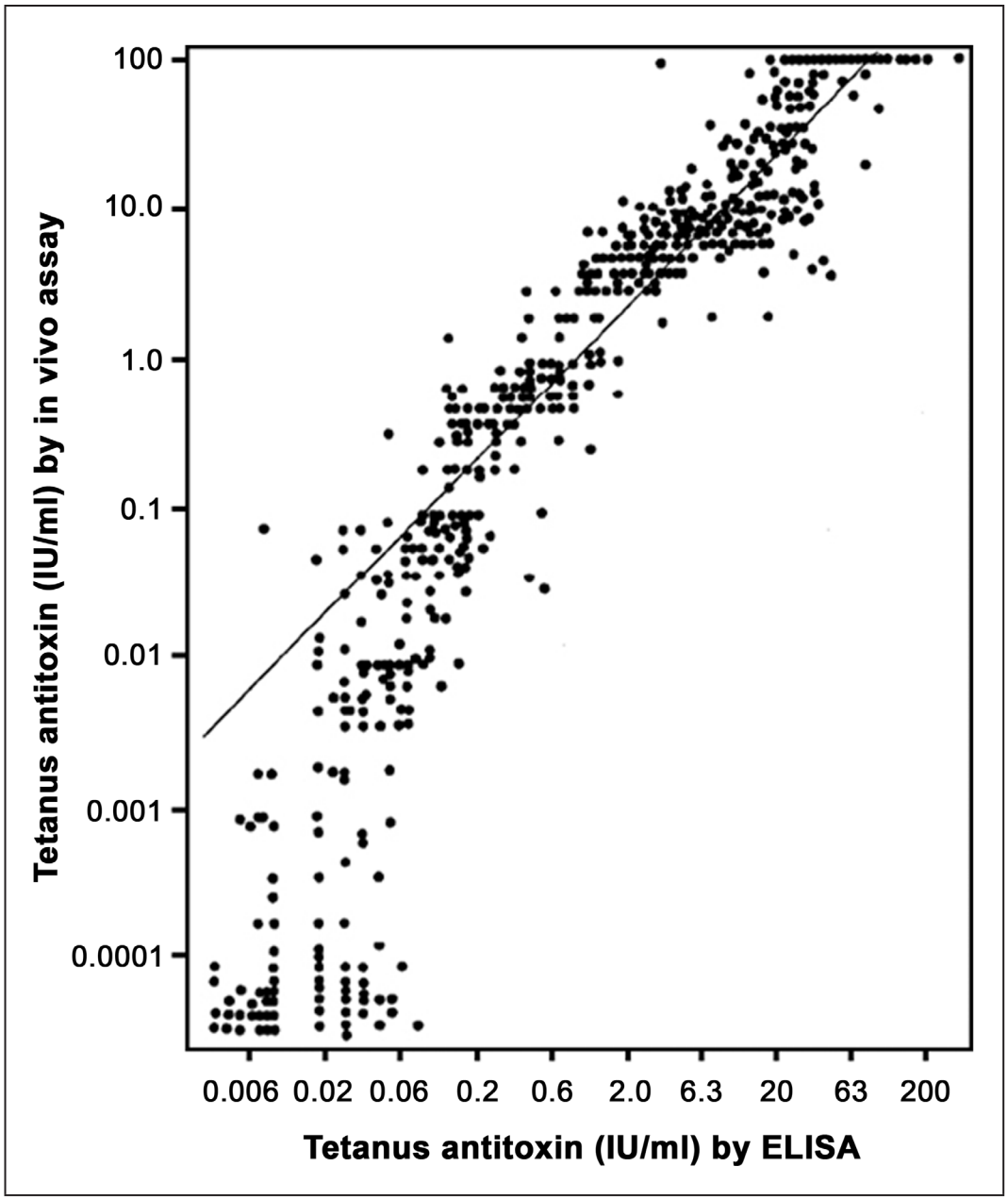
The overestimation of tetanus antitoxin levels and lack of specificity of the ELISA could be attributed to several factors, such as non-specific binding of antibody to contaminants in the antigen preparation, or recognition of non-biologically important epitopes which may be an artifact created in the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

3.2.4 Modified ELISA

A number of commercial options exist for tetanus-indirect ELISAs. The commercial ELISA tests have the same limitations as the in-house ELISA tests described above and the same associated implications in the interpretation of results (see Section 4). Two studies, one of five and the other of three commercially available kits, concluded that there were differences in sensitivity and accuracy in these tests that translate into important disparities in final results (van Hoeven et al., 2008; Perry et al., 2009). Further independent validation and comparison of commercial kits is needed to ensure comparability across studies.

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, Schou & Heron, 1987b). The competition ELISA involves mixing test sera with TT 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 thus functionality, or whether it corrects for the presence of non-specific antibodies (Simonsen, Schou & Heron, 1987b). The competition ELISA compares favorably to the in vivo neutralization assay to assess antibody levels <0.01 IU/ml (Simonsen, Schou & Heron, 1987b). A competition ELISA was used in a national serosurvey among recently pregnant women in Burundi (WHO, 1996a), but additional complexities associated with this test compared to ELISA may make it less feasible to establish in resource-limited settings (Deming et al., 2002).

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



Source: Galazka, 1993 (Original data from Simonsen, Bentzon & Heron, 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 TT to a polyclonal antitoxin by tetanus antibodies in the test sera. The ToBI assay has been subsequently demonstrated to be able to measure tetanus antibody levels below 0.01 IU/ml, making the test attractive for assessing tetanus immunity (van Gageldonk et al., 2008). ToBI has been used in national serosurveys in the Netherlands and Sweden (Böttiger et al., 1998; de Melker et al., 2000), and other reports have demonstrated the feasibility of establishing the assay in Viet Nam and Brazil (Hong et al., 1996; Sonobe et al., 2007).

A double antigen assay format has been developed which does not require any additional specialized equipment compared to the traditional ELISA (Aggerbeck, Norgaard-Pedersen & Heron, 1996; Kristiansen, Aggerbeck & Heron, 1997). Anti-tetanus antibodies in test sera are detected if bound to the solid-phase TT and a labeled TT 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, 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 persons of various ages in Australia and Turkey (Gidding et al., 2005; Caglar, Karakus & Aybay, 2005) and in assessment of the serological protection of recently pregnant women in the Central African Republic and women of reproductive age in Cambodia, demonstrating its potential for analysis of a large number of samples (Deming et al., 2002; Scobie et al., 2016).

3.2.5 Bead-based immunofluorescence assays

More recently, bead-based immunofluorescence assays have been developed which detect IgG antibodies bound to TT-conjugated microspheres (Pickering et al., 2002). These tests have been demonstrated to show good correlation to ToBI (correlation coefficient = 0.96) and double antigen ELISA (correlation coefficient = 0.91; 99% sensitivity and 92% specificity), and to have a wider dynamic range than ToBI and ELISA, reaching well below 0.01 IU/ml (Van Gageldonk et al., 2008; Scobie et al., 2016). Bead-based immunofluorescence assays are also attractive because of their ability to be multiplexed in order to measure antibodies to multiple antigens (e.g. viral, parasitic, bacterial) simultaneously from the same small volume of serum (1-5 µl, or <1/10 of the volume required for ELISA). For these reasons, the tests are increasingly used in serosurveys, as in Cambodia, Kenya, Mali, Mozambique, Netherlands, the United Republic of Tanzania and the United Kingdom (Steens et al., 2010; Wagner et al., 2012; Basta et al., 2015; Scobie et al., 2016; Scobie et al., 2017). Bead-based immunofluorescence assays for detecting tetanus IgG are not available as a commercial kit, so advanced laboratory technical assistance is needed to establish and standardize the assay. However, performing bead-based assays is similar to performing ELISAs, and capacity has been established in low-resource country settings (e.g. Kenya). These assays are proving of use in serosurveys (Scobie et al., 2017).

3.2.6 Point-of-care tests

Diagnosis of tetanus immunization status by medical interview of patients is poor and therefore a number of rapid immunoassays have been developed. The most advanced of these is the Tetanus Quick Stick (TQS) (alternatively marketed as in the United Kingdom as ProTetanus and in the Republic of Korea as SD BIOLINE tetanus). This is a rapid immunochromatographic test for use in serum, plasma or whole blood. There is limited information on the clinical benefits of TQS and the published studies had relatively small numbers of subjects – i.e. 97 (Paulke-Korinek et al., 2008), 200 (Hatamabadi et al., 2011), 299 (Stubbe et al., 2007), 988 (Colombet et al., 2005) and 1018 (Elkharrat et al., 2010). Four of these were performed on patients from emergency departments and have reported moderate sensitivity and specificity when compared to the commonly-used ELISA. The sensitivities and specificities of these studies are shown in Table 1.

Table 1: Sensitivities and specificities of TQS studies

Study	TQS setting	Threshold	Sensitivity	Specificity
Stubbe et al., 2007	Total blood tested in emergency department	0.15 IU/mL	85%	87%
Stubbe et al., 2007	Total blood tested in emergency department	0.1 IU/mL	94%	97%
Colombet et al., 2005	Total blood tested in emergency department	0.2 IU/mL	69%	98%
Colombet et al., 2005	Total blood tested in laboratory	0.2 IU/mL	84%	99%
Colombet et al., 2005	Serum tested in laboratory	0.1 IU/mL	86%	99%
Paulke-Korinek et al., 2008	Serum tested in travel clinic	0.1 IU/mL	55%	100%
Hatamabadi et al., 2011	Serum tested in emergency department	0.1 IU/mL	86%	98%
Elkharrat et al., 2010	Total blood or serum tested in emergency department	0.2 IU/mL (blood), 0.1 IU/mL (serum)	83%	97%

A study in the Netherlands compared the indication of whether tetanus post-exposure prophylaxis should be given according to the Dutch national guidance with TQS results using sera from three different emergency departments (van der Mass et al., 2016). The authors concluded that the use of TQS together with better adherence to the national guidance could prevent over-administration of TT doses and decrease the risk of being infected with tetanus as a result of more appropriate administration of anti-tetanus immunoglobulin. In France, medical interviews showed that TQS was cost-effective in patients with tetanus-prone wounds or aged 65 years or over but not cost-effective in patients with non-tetanus-prone wounds (N'Diaye et al., 2014).

In Nigeria, TQS was compared to ELISA in sera from 304 hospitalized children (Orimadegun, Orimadegun & Adepoju, 2013), showing that TQS sensitivity and specificity were 96% and 98% with a positive predictive value of 98% and negative predictive value of 96%. In Cambodia, both TQS and a second rapid test, Tetanotop, were both compared to ELISA using a threshold of 0.1 IU/mL and also to mouse serum neutralization tests using a threshold of 0.01 IU/mL (Schlumberger et al., 2015). Compared to the mouse neutralization tests, the sensitivity was good (100% for TQS and 91% for Tetanotop) but the specificity was extremely low (1% for TQS and 13% for Tetanotop).

A novel dipstick device has now been developed utilizing recombinant fragment C of the TT as antigen (Ramakrishnan et al., 2017). This device demonstrated specificity of 90% and sensitivity of 90% for whole blood and 94% for plasma at field sites in Bangladesh and Gambia when compared to a commercial ELISA using a threshold of ≥ 0.1 IU/mL.

Given the above variation in sensitivity and specificity, point-of-care antitoxin testing is currently not recommended for use in diagnosis of suspected tetanus or in serosurveys.

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, as in other efforts for diphtheria, measles and rubella. Hendriksen & Winsnes (2002) reported on an inter-laboratory comparison of ELISA and ToBI assays which demonstrated that differences were generally less than two-fold. However, comparisons of commercial ELISA tests reported large differences in sensitivity and accuracy in these tests (van Hoeven et al., 2008; Perry et al., 2009). 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 (see Table 1). When reporting results of tetanus IgG assays, the test method and cutoff used should be stated, as should the correlation with a neutralization assay or other validation process, if known. Use of the international standard serum TE-3 WHO reference serum is encouraged.

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 must consistently predict protection at an individual level and must 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 nonfunctional assay, which correlates strongly with the surrogate of protection (Borrow & Miller, 2006). Hence, the measurement of toxin-neutralizing activity in serum 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, natural immunity is not thought to occur, 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. Sneath, Kerslake & Sruby (1937) are credited with first hypothesizing that this level would be sufficient to prevent disease in humans. 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, Kerslake & Sruby (1937) noted that 13% of guinea pigs developed clinical tetanus despite antibody levels as high as 0.1 to 0.5 IU/mL. Reliable 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 *C. tetani* spores without experiencing any clinical symptoms. As it is unclear as to the level of toxin required to cause disease, 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, Kerslake & Sruby, 1937; Cowles, 1937; Wolters & Dehmel, 1938; Shumacker & Lamont, 1942; Zuger, Greenwald & Gerber, 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, Stafford & Goldman, 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.

Based on the proposed mechanism of protection of the antitoxin, the level required to prevent clinical symptoms probably relates to the severity of the infection and the level of toxin produced in the body. The difficulty in assigning a definitive level of antibody for protection is illustrated by the number of cases of tetanus that have occurred in persons with antibody levels greater than or equal to 0.01 IU/mL by neutralization assay, or 0.16 IU/mL by ELISA (Table 2). One limitation of the interpretation is that some tetanus cases had levels ≤0.2 IU/mL by ELISA, for which confirmatory testing by neutralization assay has been suggested (Simonsen, Bentzon & Heron, 1986).

Table 2: 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 and 1.0 IU/mL (54 patients had levels <0.01)	Neutralization assay (NA)
Passen & Andersen	1986	Patient had level of 0.16 IU/mL at onset[2]	ELISA
Maselle et al.	1991	7 patients had levels of 0.04-0.13 IU/mL[1,2]	ELISA
Crone & Reder	1992	3 patients had levels of 0.15–25 IU/mL (One had <0.01 IU/mL by NA)[2]	ELISA
de Moraes-Pinto et al.	1995	9 neonates had levels >0.01 IU/mL (ELISA ranges: neonates 0.07–2.83; mothers 0.28–4.81)	NA
Pryor, Onarecker & Coniglione	1997	Patient had level of 1.0 IU/mL	NA
Abrahamian et al.	2000	Patient had level of 0.16 IU/mL[2]	ELISA
Beltran et al.	2007	Patient had level of 0.22 U/mL	Not quoted[3]
Livorsi, Eaton & Glass	2010	Patient had level of 2.78 IU/mL	Bead-based immunofluorescence
Ergönül, Sözen & Tekeli	2016	Patient had level of 1.3 IU/mL 22-year-old, received TT vaccination at 14, 16 and 18 months, 2, 5 and 16 years	Not quoted[3]

1 Authors defined the level as protective by ELISA; higher cutoffs (≥0.16 IU/ml) for ELISA are suggested to reduce false positives.

2 Authors defined the level as protective by ELISA; confirmatory testing by NA is suggested for levels ≤0.2 IU/mL (Simonsen, Bentzon & Heron, 1986).

3 When reporting results of tetanus IgG assays, the test method and cutoff used should be stated to ensure comparability.

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 antibody level. This has been illustrated for pertussis and respiratory syncytial virus (RSV) (Siber, 1997), meningococci (Borrow & Miller, 2006) and pneumococci (Jodar et al., 2003). To date, such studies have not been performed for tetanus; 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. Within in vivo neutralization tests, modified ELISAs or bead-based immunofluorescence assays, concentrations at or exceeding 0.01 IU/mL are usually considered protective against disease, whereas antitoxin concentrations of at least 0.1–0.2 IU/mL are defined as positive when ELISA techniques are used for the 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 vaccination

5.1 Immune response to vaccination

As described in the 2017 WHO Position Paper (WHO, 2017a), the primary goals of immunization with tetanus-toxoid-containing vaccines are "(1) to achieve global maternal and neonatal tetanus elimination (MNTE) and (2) to ensure lifelong protection against tetanus in all people by attaining and sustaining high coverage of 6 doses (3 primary plus 3 booster doses) of tetanus-toxoid-containing vaccines (TTCVs) through routine childhood immunization schedules." Both aims require consideration of the timing of the development of immunity following primary and booster vaccination to ensure that sufficiently high levels of immunity persist.

The robustness of the immune response and the duration of immunity following TTCV administration depends on many factors, including the age at both primary and booster vaccination and the timing of vaccination doses relative to one another.

Evidence for the immune response to TTCVs among infants and children has led to the current immunization schedule recommendations outlined in the WHO Position Paper and recommendations (WHO, 2017a; WHO, 2018) and summarized in Section 5.2. Current recommendations are for three primary doses of TTCV in infancy followed by three booster doses during childhood. Evidence of robust immune responses in infants following three doses has been shown in numerous studies. For instance, studies of the pentavalent vaccines DTaP5-IPV-Hib (Pediacel) and DTaP3-IPV/Hib (Infanrix) administered at 2, 3 and 4 months of age provide evidence of ELISA geometric mean antibody concentrations (GMCs) above the minimum putatively protective level of 0.01 IU/mL at 28–42 days following administration (Grimprel et al., 2011). The more recently developed hexavalent vaccines ($DTaP_5$-IPV-HB-Hib) have been shown to induce relatively higher GMCs of 2.44 IU/mL (95% CI: 2.31, 2.59) based on ELISAs one month after the third dose when administered on a hexa-penta-hexa mixed schedule at 2, 4 and 6 months of age (Martinón-Torres et al., 2017). Factors influencing immune responses – apart from the number, timing and formulation of doses – are discussed in sections 5.4 and 7.

To illustrate the kinetics of immunity among children ≥1 year, adolescents and adults following primary and booster vaccination with TTCV, Figure 2 provides a schematic diagram of the typical response. A single dose of TT in the absence of priming induces little, if any, protection. Two to four weeks after the second dose, the mean level of tetanus antitoxin typically exceeds the minimum putatively protective level of 0.01 IU/mL. One year after the second dose, the mean antibody levels are expected to decline and may fall to the protective threshold level. After each subsequent dose of vaccine, immunity is boosted, then persists above the protective threshold for a

time, and then wanes over time. Putatively protective levels of immunity are induced by a primary series of three TTCV doses and immunity typically persists for at least 5 years. After the third dose, each additional booster dose given after at least a one-year interval increases tetanus antitoxin levels and further prolongs the duration of immunity. Immunity may persist for approximately 10 years after the fourth dose of TTCV and for at least 20 years after the fifth dose.

Eliminating maternal and neonatal tetanus requires high vaccination coverage with an adequate number of doses of TTCVs among women of reproductive age including pregnant women (WHO, 2018). Infants born to women with suboptimal levels of antitoxin may be at risk of tetanus. Evidence indicates that vaccination of pregnant women is highly effective in reducing the risk of neonatal tetanus (Demicheli, Barale & Rivetti, 2015). Section 5.2 describes the recommended vaccination schedule for women of reproductive age and pregnant women; Section 6 provides further discussion of maternal antibodies and transfer of passive immunity to neonates.

Figure 2: Schematic diagram of the antibody response to tetanus toxoid (TT) among children ≥1 year, adolescents and adults

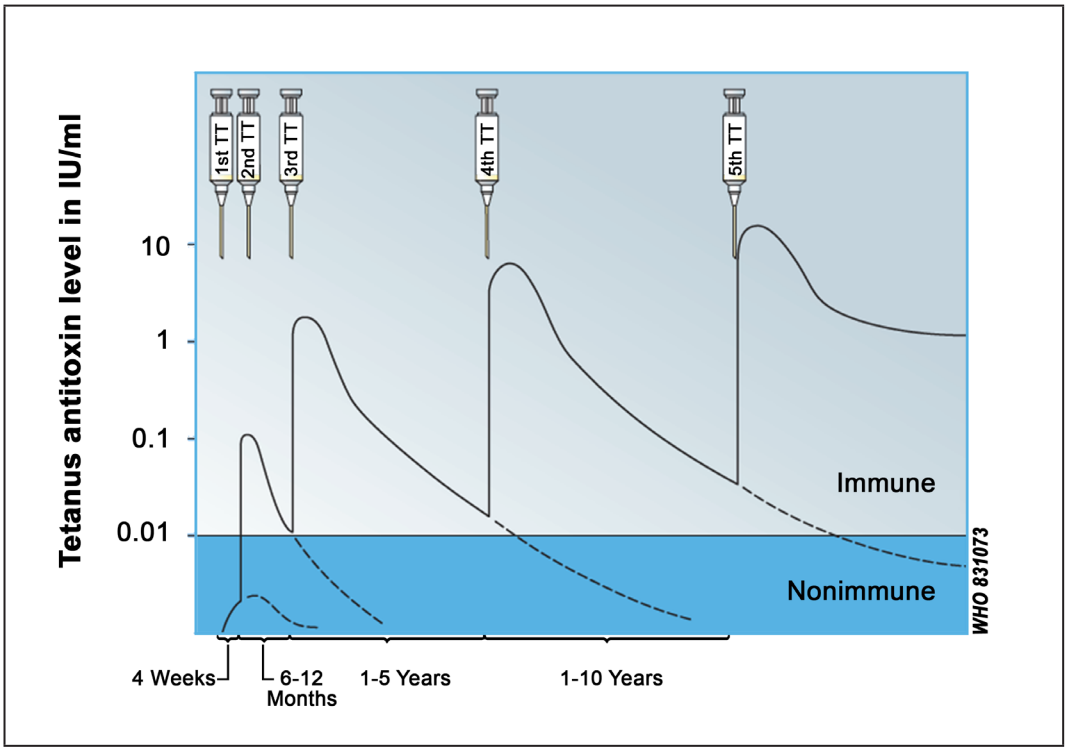


Figure reproduced from: Plotkin S, Orenstein W, Offit P, Edwards KM. Plotkin's vaccines, seventh edition. New York (NY): Elsevier; 2017: Chapter 58, Tetanus toxoid, p. 1071, reproduced by permission.

"Five properly spaced doses [given to children ≥1 year, adolescents and adults provides] protection lasting at least 20 years and probably substantially longer for most recipients. (Threshold of protection of 0.01 IU/mL applies to values measured by in vivo neutralization, modified ELISA [or bead-based immunofluorescence]; for standard ELISA, protection is [usually defined between 0.10–20 IU/mL, based on the assay])." (Modified source: Borrow R, Balmer P, Roper MH. The immunological basis for immunization. Module 3: Tetanus (update 2006). Geneva: World Health Organization; 2007.)

5.2 Duration of immunity following various immunization schedules

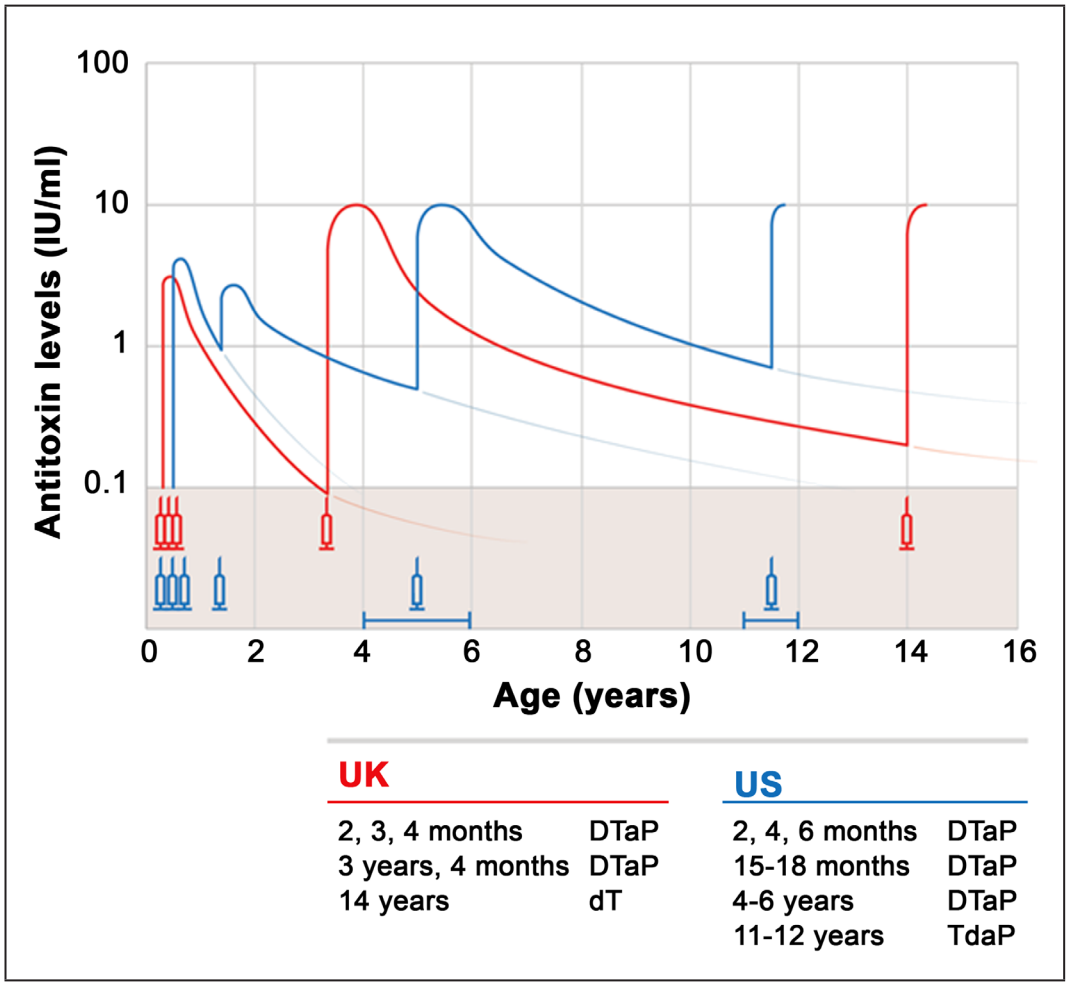
An understanding of the duration of immunity induced by primary and booster immunization has important implications for the recommended number and timing of doses needed to sustain lifelong immunity.

Serological data from the United Kingdom and the United States of America (USA) illustrate antibody profiles after two different vaccination schedules. Figure 3 presents a schematic diagram of the typical response using the example of the recommended DTaP and Td vaccinations in the United Kingdom and the DTaP and TdaP vaccinations in the USA. It should be noted that both countries currently recommend combination vaccines rather than DTaP alone. Consult the most up-to-date regulatory authority to find out more about the specific vaccination formulations recommended in a given country.

In the United Kingdom, DTaP is recommended at 2, 3 and 4 months of age, followed by a school-entry DTaP booster at 3 years 4 months and dT at 14 years of age (PHE, 2013). Although antibody levels decline after the primary series in infancy, there is an excellent response to the booster dose at the age of school entry and antibody levels persist at least until 14 years of age, when provision of another booster dose results in a rapid and robust increase in antibodies.

In the USA, DTaP is recommended at 2, 4 and 6 months, followed by DTaP boosters at 15–18 months and 4–6 years, and TdaP at 11–12 years of age (CDC, 2018a). Evidence suggests that, following the initial peak in antibody levels after the first three primary doses, booster vaccination at 15–18 months of age induces another antibody peak. Children receiving a booster dose in the second year of life have been shown to have higher antibody levels in the years leading up to school entry than those who do not receive this dose (WHO, 2017a). However, by the age of school entry, antibody levels wane again. The response to the second and third booster doses in childhood and early adolescence results in a rapid and robust increase in antibodies. Previous studies have suggested that, during the teenage years, the antibody profiles among the United Kingdom and United States teens are similar. While providing a DTaP booster during the second year of life in the USA may induce higher tetanus antibodies during the toddler and preschool years than schedules without a booster, the 2006 introduction of MCC/Hib-TT in the United Kingdom, recommended at 12 months of age, closed the immunity gap during those ages (Wagner et al., 2012); both schedules induce robust immune responses among school children and lay the foundation for long-lasting protection during adolescence and adulthood.

Figure 3: Schematic diagram of the typical, relative responses following two different infant and childhood vaccination schedules – DTaP and dT vaccinations in the United Kingdom and DTaP and TdaP vaccinations in the USA.



Sources: Voysey et al., 2016; Vergara et al., 2005; Swartz et al., 2003; Lin et al., 2003; Burrage et al., 2002; Scheifele, Guasparini & Lavigne, 1999; Ramsay et al., 1993

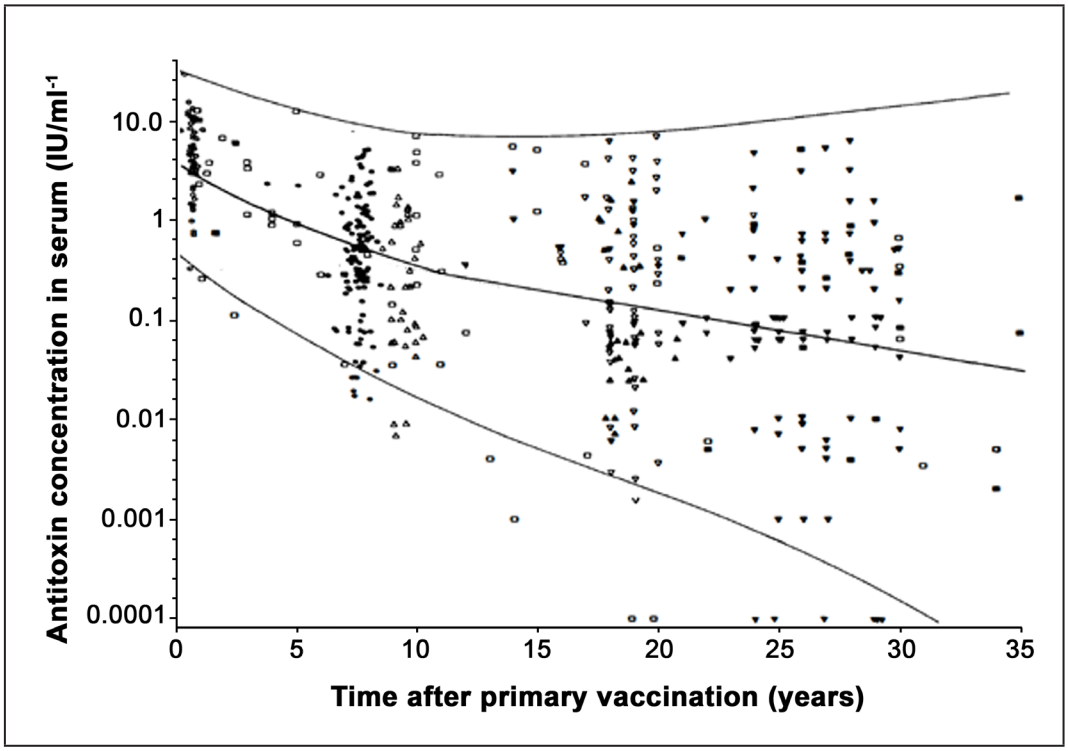
Assays: ELISA except for Ramsay et al. = RIA.

Note: The United Kingdom introduced MCC/Hib-TT in 2006, recommended at 12 months of age which, evidence suggests, closed the immunity gap for preschool-aged children.

For countries implementing the Expanded Programme on Immunization (EPI) schedule, WHO recommends an accelerated schedule of three infant doses beginning as early as 6 weeks of age, with at least 4 weeks between doses and completion of all doses by 6 months of age if possible (WHO, 2018). Three additional booster doses of TTCV are recommended at ages 12–23 months, 4–7 years and 9–15 years (with at least 4 years between doses) (WHO, 2017a). Data available on the duration of immunity following immunization in the EPI schedule often have limitations such as the design of the study (cross-sectional), the type of assay performed, the appropriateness of the data analyses, and whether ages at vaccination or duration since last vaccination have been documented. Consequently, it can be difficult to interpret data on duration of antibody levels following immunization under the EPI schedule. Evidence from some resource-limited countries suggests that immunization gaps result in inadequate protection among school children aged 5–14 years and adult males who have not been the focus of vaccination efforts which have prioritized protection of young children and women of reproductive age (WHO, 2017a; Scobie et al., 2017).

Ensuring sufficiently high immunity among women of reproductive age, especially pregnant women, is critical to achieving and sustaining MNTE. The WHO-recommended vaccination schedule for a pregnant woman varies according to her vaccination history. Pregnant women who received a complete six-dose series of TTCV begun during infancy, or a five-dose series if begun at or following 1 year of age and completed by reproductive age, will have sufficient antibody levels to protect themselves and their newborns. In countries where MNT remains a problem, pregnant women without a documented tetanus vaccination history should receive at least two TTCV doses — one as soon as possible during pregnancy, and again 4 weeks after the initial dose and at least 2 weeks before the due date. A third dose given at least 6 months later ensures 5 years of protection, and a fourth and a fifth dose should be given with intervals of at least 1 year between doses in order to ensure long-term protection (>20 years). Pregnant women who received a documented three doses of DTP during childhood are recommended to receive three additional doses – two doses during pregnancy and a third dose 1 year later (WHO 2017). Pregnant women who received four DTP doses as children should receive one dose during the first pregnancy and a second dose 1 year later (WHO, 2017a). Supplementary immunization activities targeting women of reproductive age in high-risk areas are completed where necessary to provide three TTCV doses, irrespective of previous vaccination status, delivered in three rounds, with an interval of at least 4 weeks between the first and second doses, and at least 6 months between the second and third doses (WHO, 2017a).

Figure 4: Duration of protection in Danish subjects following three doses of TTCVs and no revaccination



Reproduced from Simonsen et al., 1987a.

Each data point represents an individual immunized with one of the described immunization schedules (n = 439).

Immunization schedule relevant to study participants: Infants – either three doses of DT (12 Lf TT) at 5, 6 and 15 months, or three doses DT-Pol (Polio) (7Lf TT) at 5, 6 and 15 months. Adults – 3 doses of TT (12 Lf) at 0, 1 months and 1 year apart.

Symbols correspond to different age groups: open circle = 2 years; filled circle = 8–9 years; open triangle pointing up = 10–11 years; open square = 50–83 years; filled triangle pointing up = 19–22 years; open triangle pointing down = 19–25 years; filled square = 30–69 years; filled triangle pointing down = 25–30 years.

Data from studies conducted in Denmark (Simonsen et al., 1987a) at a time when TTCVs were administered at 5, 6 and 15 months (much later than the schedule currently recommended by WHO) using vaccines that contained a higher concentration of antigens than currently-recommended vaccines (see Figure 4, including footnotes) demonstrate the longevity of the immune response and the persistence of immune memory as evidenced by the response to revaccination many years later. Analysis of the antibody levels of 439 participants who received three doses of TT at these later ages without being revaccinated suggested that antibody levels persisted above 0.1 IU/mL (by ELISA) up to 25 years after the last immunization (Figure 4) (Simonsen et al., 1987a). The authors concluded that this schedule of primary immunization in infancy gives approximately 5 years of protection and that revaccination within 5 years of the last dose induces immunity for up to approximately 20 years. Data from a cross-sectional study in the Netherlands (de Melker et al., 2000), where six doses of TT are given in childhood and the final dose is provided at 9 years of age, also demonstrated that at approximately 20 years after the last dose the geometric mean antibody level was 0.44 IU/mL by the ToBI assay.

Historically, boosters were recommended every 10 years in the USA because of concerns about shorter-lived immunity with fluid TT rather than adsorbed, and because of concerns regarding variation in the potency of either preparation (Levine et al., 1966). Booster responses may vary and a pronounced dispersion of antibody levels can be expected with time following a primary series of immunizations (as seen in Figure 4); hence, regular boosters were used to maximize protection for a high proportion of the population. Many countries, including the USA, still recommend that boosters be routinely administered every 10 years during adulthood (CDC, 2018b), whereas some countries, such as the United Kingdom, do not recommend any further doses following the five doses received as a child and adolescent except, if appropriate, in wound management or for travel to certain settings if the most recent TTCV was received more than 10 years previously (NHS Choices).

Uncertainty remains regarding the need for routine booster doses every 10 years due to lack of appropriate evidence on which to base policy. Several studies have provided evidence that immunity persists beyond 10 years (a duration of 20–30 years has been suggested), though it is important to consider the timing and age of vaccination in those studies (de Melker et al., 2000; Simonsen et al., 1987a; Hammarlund et al., 2016; Embree et al., 2015). Although the half-life of tetanus immunity has been estimated to be approximately 11 years (Amanna, Carlson & Slifka, 2007), other evidence suggests that antibody levels decline significantly over a 10-year period following the peak at 1 month post-vaccination (Tomovici et al., 2012). Further research is needed to determine whether there are clinical implications following such a decline. In the absence of recommendations for adult TTCV boosters or when recommendations are made but uptake is suboptimal, a proportion of older adults, which will vary by setting, may experience significant waning of immunity resulting in antibody levels below putatively protective thresholds (Theeten et al., 2011; Launay et al., 2009; Wagner et al., 2012; Maple et al., 2001). In some cases, a higher proportion of cases among older persons has been reported, as in the countries of the European Union (ECDC, 2015). The need for and timing of routine booster doses in adulthood in a given setting is likely to depend on the risk of disease, the recommended infant, childhood and adolescent vaccination schedule that applies to each age cohort, and the vaccination coverage and series completion rates during the relevant time period.

The magnitude of the response to a booster dose of TT can depend on the time since last vaccination and circulating antibody levels. It has been widely reported that the higher the pre-booster antibody level, 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 appear to provide additional short-term or long-term protection. Therefore, immunization schedules should be appropriately spaced to provide the optimal timing for booster vaccinations. 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. Currently, evidence is lacking to evaluate the kinetics and duration of the immune response to a booster dose administered more than 10 years after the 6–10–14 weeks infant schedule. Further research is needed in this area to better inform policy (Bracebridge, Crowcroft & White, 2004; WHO, 2016c).

The kinetics of the response to a tetanus booster are important for many reasons, including for recommendations for managing patients with tetanus-prone wounds. For the management of patients with tetanus-prone wounds, it is important to note that the median period of incubation to onset of tetanus has been reported as 7 days (range 0–112 days) (Pascual et al., 2003). A measurable increase in antibody titre following a booster dose has been detected after 4 days (Turner, Stafford & Goldman, 1954; Simonsen et al., 1987c), but in general it takes 6–7 days to reach substantial antitoxin levels (Looney et al., 1956; McCarroll, Abrahams & Scudder, 1962; Turner, Stafford & Goldman, 1954). It is thought that maximum levels are reached by 2 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 incubating tetanus if antitoxin levels are low, but it will provide long-term protection against future *C. tetani* exposure.

In summary, a primary series of 3 TTCV doses in infancy plus a booster during the second year of life will provide 3–5 years of protection. A further booster dose (e.g. in early childhood) will provide protection into adolescence, and another booster during adolescence will induce immunity that lasts through much of adulthood, thus protecting women through their childbearing years. Booster responses after 6 TTCVs can be elicited after intervals of 20–30 years.

5.3 Serological surveillance of tetanus toxoid immunity

Because recovery from tetanus does not confer natural immunity, detectable anti-tetanus antibody levels can be attributed to: 1) active immunity conferred by past vaccination, 2) passive immunity obtained through transfer of maternal antibodies (short-lived), or 3) passive immunity obtained through receipt of tetanus immunoglobulin (Ig). As a result, serological surveys of anti-tetanus antibody levels can provide insight into population-level patterns of immunity and can demonstrate the impact that various vaccination schedules have had on age-specific immune responses. However, serological surveys are limited in that they cannot distinguish between the number of doses of TTCVs received, the time since vaccination and many other factors related to the kinetics of immunity, although they can identify gaps in immunity in specific groups. Multiple serosurveys have been undertaken in various settings, at different time points, among various age groups and among persons following various vaccination

schedules, making it very difficult to compare results drawn from such diverse settings. A complete summary of the literature is beyond the scope of this publication, but further evidence can be reviewed in detail as part of a recent SAGE Working Group report (WHO, 2016b).

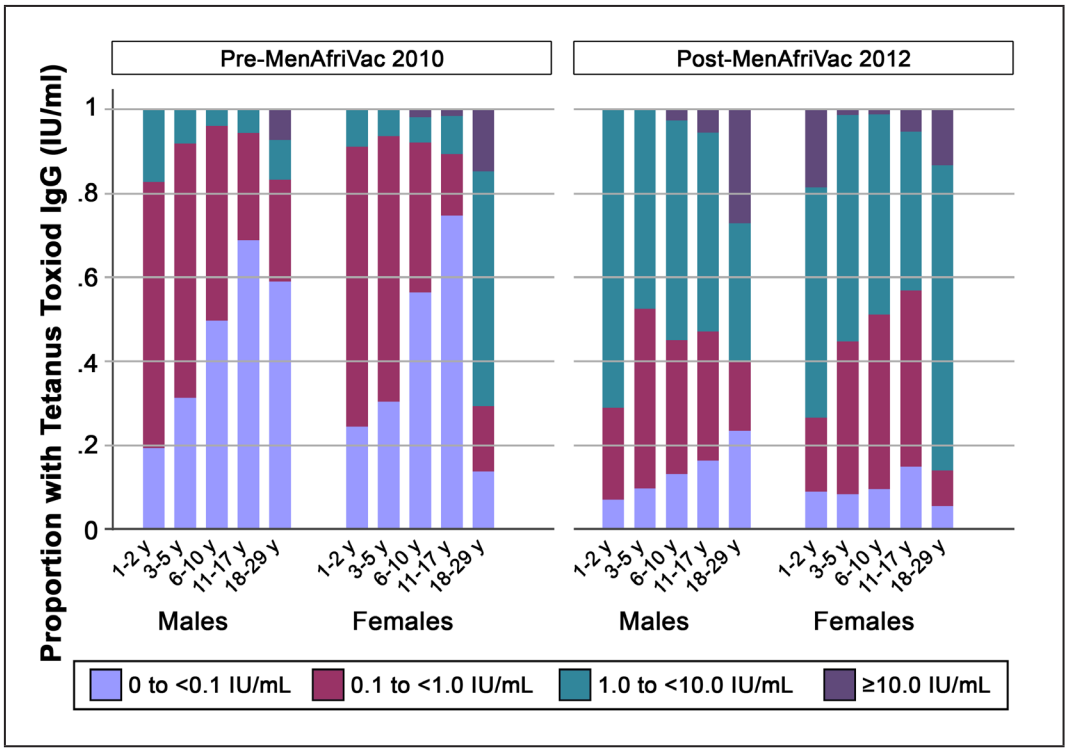
Most tetanus serosurvey data come from high-income countries with established immunization programmes, many of which have achieved high coverage during infancy, childhood and adolescence, with variable booster coverage in adulthood (offered alongside wound care or as part of routine prevention). Trends in antibody levels based on serosurveys in these settings suggest that, typically, higher antibody levels are observed in younger age groups, with lower antibody levels seen among older age groups (Symeonidis et al., 2003; Redwan & Al-Awady, 2002; Ergonul et al., 2001; De Melker et al., 2000; Stark et al., 1999; Yuan et al., 1997; Stroffolini et al., 1997). Evidence from several studies suggest higher susceptibility among older adult females than among males (Symeonidis N et al., 2003; de Melker et al., 2000; ECDC, 2015; Steens et al., 2010; Gidding et al., 2005; McQuillan et al., 2002).

A number of tetanus serosurveys from low-income countries have provided important evidence on the impact of vaccination programmes. For instance, a cross-sectional study from Kenya illustrated the distribution of tetanus immunity in a population where the EPI schedule had been implemented since 1983, with an initial catch-up campaign to immunize young children and pregnant women. Antibody levels were higher for children aged 1–7 years who would have been immunized under the EPI schedule or catch-up campaign, but lower in older children and adolescents (8–17 years). Higher antibody levels were observed in women of reproductive age due to immunization efforts (Kurtzhals et al., 1997). More recent evidence from Kenya, Mozambique and the United Republic of Tanzania revealed higher seroprevalence among 5–14-year-olds compared to 1–4-year-olds only in Mozambique where two booster doses were recommended during the school years; in Kenya and Tanzania such booster doses were not offered (Scobie et al., 2017). Another recent study was conducted among persons aged 1–29 years in Bamako, Mali, where TTCV is provided at 6, 10 and 14 weeks of age and TT vaccination is provided during pregnancy, but no TTCV booster doses are given to either sex as part of routine immunization. In this study, the proportion susceptible to tetanus (antibody levels below 0.1 IU/ mL) was highest among 11–17-year-old males and females and 18–29-year-old males, with lowest susceptibility among 18–29-year-old females (Figure 5) (Basta et al., 2015). These trends were observed in 2010 prior to the introduction of the PsA-TT (MenAfriVac) mass vaccination campaign which targeted all persons aged 1–29 years. Two years after the mass vaccination campaign, evidence suggested that TT antibody levels had been boosted among all age groups eligible for PsA-TT vaccine (Figure 5) (Basta et al., 2015).

These data highlight that serosurveys require knowledge of the changes in immunization programmes over time and changes in the coverage for each birth cohort to allow for appropriate interpretation. In addition, cross-sectional serosurveys capture a snapshot of immunity which needs to be updated as efforts to improve vaccination coverage increase over time.

Serological surveys also illustrate the potential for appropriately scheduled primary series and boosters to provide high antibody levels to protect women against tetanus throughout their reproductive years. For instance, serological surveys reported in the literature (Maple et al., 2001; McQuillan et al., 2002; Gidding et al., 2005) provide evidence that seroprotection among women remains consistent at 80% from a young age until about the age of 40 years before decreases in seroprotection are observed. These trends vary by country, age cohort, and immunization schedule and may change over time, but they suggest that a complete primary series of immunizations and subsequent boosters in childhood and adolescence induces protective antibody levels well into adulthood, protecting women throughout their childbearing years and subsequently protecting their newborns (see Section 6 for an in-depth discussion of placental passage of tetanus antitoxin). Serosurveys are an important tool for monitoring progress towards MNTE and can identify immunization gaps and priorities for strengthening immunization activities among target groups (Scobie et al., 2016; Deming et al., 2002; WHO, 2011).

Figure 5: Distribution of TT IgG antibody concentrations among repeated cross-sectional surveys of randomly selected, age-stratified samples of residents of Bamako, Mali, in 2010 prior to the PsA-TT (MenAfriVac) mass vaccination campaign, and in 2012 after the PsA-TT campaign targeting all 1–29-year-olds



Source: Basta et al., 2015

Note: Age shown in both panels of the figure is age in December 2010.
Reprint of an Open Access article distributed under the terms of the Creative Commons Attribution License.

5.4 Factors influencing the immune response to tetanus toxoid

Many factors can influence the immune response to TTCVs. Two particular conditions that may influence the immune response to TT are malaria infection and human immunodeficiency virus infection/acquired immunodeficiency syndrome (HIV/AIDS). In many areas where MNTE has not been achieved, the prevalence of these infections is high.

The response to TT vaccination among women infected with malaria during pregnancy is similar to that in non-pregnant healthy adults (Brabin et al., 1984). However, maternal transfer of tetanus antibodies to newborns was reduced both among women who had active, chronic or past placental malaria, and among women infected with HIV (Cumberland et al., 2007). Section 6 includes further discussion of malaria during pregnancy and additional factors affecting placental transfer of antibodies. Studies of children infected with malaria have reported a decreased response following one or two doses of TTCVs in children with parasitaemia from acute malaria compared to non-parasitaemic controls (Edsall et al., 1975; Greenwood et al., 1972). However, studies have suggested that malaria chemoprophylaxis does not impair antibody responses to TTCVs in children (Gilles et al., 1983; Monjour et al., 1982; Rosen & Breman, 2004). Dietz et al (1997) conducted a systematic review, including the studies noted above, and concluded that complete evaluation of the impact of both concurrent malarial infection and the impact of antimalarial therapy on immune responses to TTCVs requires analysis of infected (both treated and non-treated) individuals and non-infected individuals; however, conducting a study that withheld antimalarials from infected individuals would be unethical.

Inactivated vaccines are safe for use in immunocompromised individuals, and all eligible persons, regardless of immunocompetence status, are recommended to receive TTCVs. WHO recommends that all children infected with HIV should be vaccinated to protect against tetanus in accordance with guidelines established for children not infected with HIV (WHO, 2018). Evidence suggests that infants infected with HIV during the perinatal period develop sufficient responses to infant TTCV doses, though response may decline as HIV progresses (Ryder et al., 1993; Borkowsky et al., 1992; Roper et al., 2017; von Reyn, Clements & Mann, 1987). Among HIV-positive children and adults, the proportion of HIV-positive persons who respond to TTCVs is similar to the proportion of HIV-uninfected individuals, though responses are often lower and may wane more quickly (Moss, Clements & Halsey, 2003). Moss and colleagues reviewed the literature and found that 40–100% of individuals not infected with HIV develop protective levels of tetanus antitoxin following primary immunization in infancy (Moss, Clements & Halsey, 2003). HIV-infected children respond well to booster immunization, with 74–90% exhibiting protective antibody levels following receipt of a booster dose at various respective ages and times since the primary infant series (Borkowsky et al., 1992; Rosenblatt et al., 2005; Melvin & Mohan, 2003).

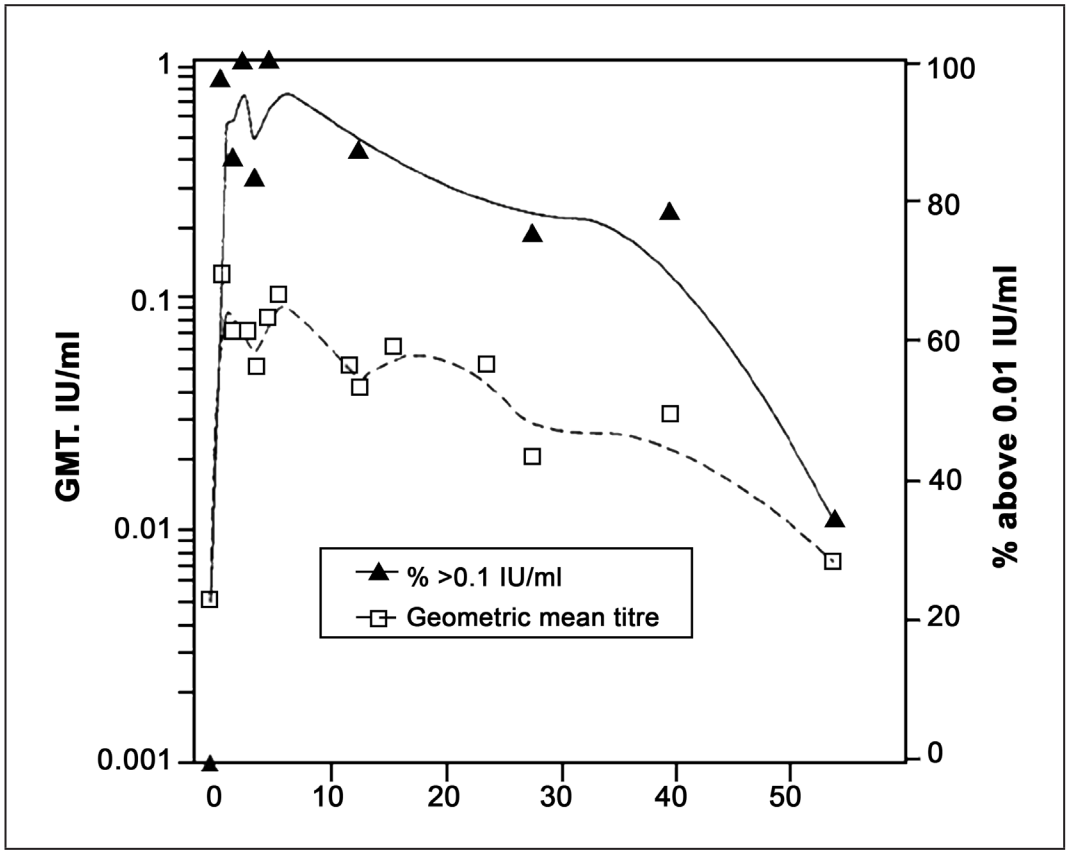
A seroprevalence study among migrants infected with HIV from sub-Saharan African countries living in France found relatively low (58–79%) seroprotection (>0.1 IU/mL, ELISA) prior to travel, although their vaccination history was unclear. A small proportion of these individuals were vaccinated during their pre-travel consultation, prompting seroprotection levels to increase significantly in both males and females after travel (Mullaert et al., 2015).

6. Placental passage of tetanus antitoxin

6.1 The placenta as a selective organ

Vaccination in pregnancy has been shown to be safe and effective. A study in Papua New Guinea showed that 78% of women immunized during pregnancy with two doses of adsorbed TT containing 10 Lf had antitoxin levels above 0.01 IU/m1 for at least three years as measured by neutralization; the mean antitoxin level was about 0.03 IU/ml (Figure 6). Tetanus antitoxin transferred from immunized mother to fetus provides transient protection of the newborn infant from tetanus. Since there is no naturally-acquired immunity to tetanus, passive protection of the infant is dependent on vaccination of the mother either before or during pregnancy. The human placenta regulates the transfer of antibodies from mother to fetus in a selective manner; transplacental transfer of tetanus antibodies is largely restricted to IgG. Fetal IgG antibody levels rise progressively in the last trimester and are therefore lower in babies born prematurely. In a study in Gambia (Englund, 2007) full-term adequate weight infants had higher tetanus antibody concentrations than their mothers, with similar levels for mothers and full-term low-birth-weight infants, and lower levels in infants born prematurely than in their mothers (Okoko, Wesumperuma & Hart, 2001).

Figure 6. Geometric mean concentration 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).

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

For women who receive their first dose of TT in pregnancy, the ratio of antitoxin in maternal serum to antitoxin in cord serum depends on the intervals between TTCV doses and the interval between the last dose and delivery. Longer intervals between the first two TTCV doses increase the height and duration of the immune response (Table 3). The cord/maternal ratio of tetanus antibodies increases as the interval between the second dose and delivery is increased (Stanfield, Gall & Bracken, 1973). These data strongly support the policy of integrating maternal tetanus vaccination into a broader package of antenatal care in resource-poor settings (Lincetto, Gomez & Munjanja, 2006). Where multiple antenatal contacts can be achieved, it is important to start immunization as early as possible in the pregnancy to ensure adequate intervals between doses and between the second dose and delivery.

However, pregnant women in hard-to-reach populations may not report to health centres until pregnancy is well advanced and little time remains to give two properly-spaced TTCV doses before delivery. Lack of adherence to the recommended minimal time intervals between TTCV doses diminishes the possibility of effective transfer of a significant amount of antibody from the mother to the fetus. 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 3: Antibody levels obtained in the umbilical cord blood of women who received two doses of TT (purified adsorbed) during pregnancy, shown by levels found and interval between the doses

Interval between toxoid doses (weeks)	No. of samples tested	% distribution of antibody levels (IU/mL) in cord sera tested by neutralization assay		
		>0.01	>0.1	>1.0
4–8	238	70.6	37.0	8.4
9–12	210	81.1	62.4	15.7
13–16	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, and Suri & Rubbo, 1961).

6.3 Factors influencing the placental transfer of antitoxin

Findings from studies on the effect of placental malaria infection on transplacental transfer of tetanus-specific antibodies have varied. In a study in Gambia in 1997, in which malaria infection was assessed by measuring blood parasitaemia in mothers (Okoko et al., 2001), no effect was observed on the transplacental transfer of tetanus antibody. Similar findings were reported in a study performed in 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 parasitaemia 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 lower antibody levels in neonates associated with active chronic and past placental malaria infections, but not active acute malaria infection (Cumberland et al., 2007).

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, Cumberland et al., 2007).

6.4 Interference between passive antibodies and the development of active immunity to tetanus

The rate of decrease of maternal tetanus antitoxin in the infant during the neonatal period (Sangpetchsong et al., 1985) is similar to that for antibodies against *Neisseria meningitidis* serogroup A, *Haemophilus influenzae* type b (Hib) and group B *Streptococcus* induced by polysaccharide vaccines given to mothers during pregnancy (Amstey et al., 1985; Baker et al., 1988; McCormick et al., 1980). For tetanus antitoxin, the half-life of maternal antibody was found to be 43–57 days (Sarvas et al., 1993).

When pregnant women receive a TdaP CV to prevent neonatal tetanus and thereby to prevent infant pertussis, infants will have high levels of maternally-acquired tetanus antitoxin at the time of primary immunization. Such passive immunity could suppress the development of active immunity following early administration of DTP vaccine. One study in the Philippines showed tetanus antibody levels in infants aged 6 weeks were positively correlated with the number of TTCV doses received by the mother during pregnancy, while antibody levels after receipt of the primary series of three DTP doses were negatively correlated with the number of maternal doses (Nohynek et al., 1999). Although this finding suggests that high levels of transplacentally acquired antibodies can reduce the infant's response to DTP under the EPI schedule, the authors note that the clinical and public-health significance is unknown since all children were observed to maintain protective antibody levels up to 9 months of age, at which time a booster dose was given (Nohynek et al., 1999).

More recently, studies of the effect of maternal immunization with a TdaP vaccine on infants' responses to immunization have been conducted in a high-income country setting, largely to assess potential blunting of the pertussis responses. Two studies have assessed the impact of maternal TdaP on infants' tetanus antibody responses after a 2–3–4-month primary schedule and found no evidence of a reduction when compared with historical or contemporary control infants with unvaccinated mothers (Ladhani et al., 2015; Maertens et al., 2016). A study in the USA, using a 2–4–6-month infant schedule, also found no evidence of reduction in tetanus responses in infants born to mothers vaccinated with TdaP (Munoz et al., 2014).

Even without a maternal TdaP immunization programme, the majority of women of reproductive age in high-income countries will have protective tetanus antitoxin levels as a result of longstanding immunization programmes with primary and booster TTCV doses (Munoz et al., 2014; Gall, Myers & Pichichero, 2011; de Voer et al., 2009; Sauerbrei et al., 2004). Therefore, most infants in these high-income country settings are born with protective antitoxin levels. With a half-life of 1–2 months (Sarvas et al., 1993), maternal tetanus antibody levels in infants may still be relatively high by 2 months of age, at which point the first TTCV dose is usually administered (Munoz et al., 2014, Ladhani et al., 2015, Maertens et al., 2016). A meta-analysis that assessed the relationship between 1) maternal antibodies to tetanus in mothers in high-income country settings who did not receive a TTCV in pregnancy and 2) infants' responses to primary immunization, found evidence of a small inverse correlation between infant tetanus antibody levels after the third DTP dose and maternal antibody levels (13% lower geometric mean concentration for every 2-fold increase in maternal antibody level) (Voysey et al., 2017). The effect did not persist after a booster immunization. Also, despite the lower post-primary response in infants with higher pre-existing maternal antitoxin, mean titres were still well above the minimal level of protection 6 months after completing the primary schedule when given at 2, 3 and 4 months of age (Booy et al., 1992; Ramsay et al., 1993).

6.5 Effect of maternal tetanus antibodies on infants' response to polysaccharide vaccines conjugated to tetanus toxoid

Studies of the effect of maternal tetanus antibody levels on infants' responses to TT-conjugated Hib, pneumococcal and meningococcal vaccines have shown little evidence of an inhibitory effect. In a United Kingdom study of infants whose mothers received TdaP in pregnancy, there was a suggestion of an enhancement of infants' Hib responses (Ladhani et al., 2015). An enhancement of Hib responses in infants with high levels of maternal tetanus antibody was also shown in a study in Finland after the second dose of Hib-TT given at 3–4 months of age (Kurikka et al., 1996). In a study in the Philippines, maternal tetanus antibodies had no effect on responses to Hib vaccine, whether conjugated to TT, CRM_{197} or OMP (Nohynek et al., 1999).

For meningococcal vaccines, there is evidence that maternal vaccination with TdaP may enhance infants' responses to a serogroup C tetanus-conjugated vaccine (Ladhani et al., 2015). For pneumococcal conjugate vaccines, only serotype 18C in PCV10 is conjugated to tetanus; evidence suggested a 6% reduction in geometric mean antibody levels to this serotype in PCV10 for a doubling of maternal tetanus antibody levels (Voysey et al., 2017).

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 TTCV have been convincingly demonstrated in many field trials and in hospital-based studies. Randomized controlled trials assessing efficacy of TTCV against non-neonatal tetanus have not been conducted, although there is compelling ecological evidence of the impact of national tetanus immunization programmes in reducing tetanus cases in the population (CDC, 2011). Despite the lack of randomized, controlled clinical trials for non-neonatal tetanus outcomes, the results of field effectiveness studies of maternal vaccination against neonatal tetanus can be considered as strong evidence that vaccination with TT is a highly effective intervention.

The ability of maternal TT immunization during pregnancy to prevent neonatal tetanus was first studied in a non-randomized controlled trial in New Guinea in 1959. Vaccine effectiveness of non-adjuvanted plain TT was 94% for three doses and 65% for two doses, with no protection from one dose (Schofield, Tucker & Westbrook, 1961). High efficacy of at least two maternal doses of TT was subsequently confirmed in a double-blind, controlled field trial in a rural area of Colombia in the 1960s in which there were no fatal tetanus cases among neonates whose mothers received two or more doses of adsorbed TT in pregnancy compared with a rate of 78 per 1000 live births in infants of unvaccinated mothers (Newell et al., 1966; Newell et al., 1971). A reduction in neonatal tetanus mortality following the implementation of programmes to immunize women of reproductive age, and especially pregnant women, has also been observed in multiple countries, including Bangladesh (Black, Huber & Curlin, 1980; Rahman et al., 1982), Haiti (Berggren et al., 1983), Mozambique (Cliff, 1985a; Cliff, 1985b), Namibia (WHO, 2002), South Africa (Vandelaer et al., 2003), Sri Lanka (WHO, 1982) and Zimbabwe (WHO, 2001). A systematic review of randomized, controlled clinical trials and observational studies in 2010 concluded that immunization of pregnant women or women of reproductive age with at least two TT doses reduced mortality from neonatal tetanus by 94% (95% confidence interval (CI) 80–98%) (Blencowe et al., 2010).

7.2 Reported "failures" of tetanus toxoid immunization

TT is one of the most reliably immunogenic antigens used in current vaccines although, as with any vaccine, it is not 100% effective. Clinical cases of tetanus in children and adults have been reported despite previous receipt of TTCV. There is no clear pattern associated with these cases with respect to immunization. In some cases, the lack of protection can be explained by inaccurate immunization histories, inappropriate vaccination schedules, use of low-potency vaccines or improper storage of vaccines. It is also important to note that emergency vaccination at the time of exposure may not by itself be enough to prevent clinical tetanus because the tetanus incubation period may be too short and the increase in toxin concentration may outpace the formation of antitoxin from the response to a booster dose in a previously vaccinated person; one dose provided to a previously unvaccinated person would be predicted to be completely ineffective. However, there are a few well-documented cases of clinical tetanus occurring despite "protective" levels of antitoxin antibody (see Table 2 in Section 4). From these case reports, there is usually evidence that the clinical course of the illness is attenuated by prior immunization (Luisto & Iivanainen, 1993; Lodha et al., 2000).

Rare cases of neonatal tetanus will still occur despite a 94% efficacy of two maternal doses of TT in pregnancy. As maternal immunization coverage rises, the proportion of cases in newborns whose mothers have been immunized will inevitably increase. Table 4 shows that, although neonatal tetanus can occasionally occur despite maternal immunization, the vast majority of cases occur in mothers who are either unvaccinated or inadequately vaccinated. In Nigeria, neonatal tetanus was observed in six infants whose mothers had received ≥2 TT doses during their last pregnancy and where the antibody levels of both mothers and infants were expected to confer protection (mean antibody level by ELISA: 0.70 and 1.02 IU/mL for newborns and mothers, respectively) (de Moraes-Pinto et al., 1995). The reasons why neonatal tetanus occurred in these infants are unclear but could have included heavy tetanus spore contamination of the cord resulting in particularly large quantities of toxin production. It is important, therefore, that the elimination of neonatal tetanus does not rely solely on maternal TT vaccination but that this is combined with improved clinical practices and health education activities to ensure clean delivery and hygienic cord practices.

Table 4: Tetanus toxoid (TT) immunization history of mothers whose infants developed neonatal tetanus, based on hospital data

Country	Reference	Number of neonatal tetanus cases studied	Maternal history: number of TT doses			
			0	1	2	3
Angola	Grudeborn, 1987	199	188	0	11[1]	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[2]
India	Deivanayagam, Nedunchelian & Kamala, 1991	19	13	0	3[3]	3[3]
India	Ghosh, 1990	30	21	5	4	0
India	Kumar et al., 1988	385	363	0	22[4]	0
India	Mathur et al., 1980	50	50	0	0	0
India	Verma, Dhanwade & Singh, 1989	76	49	5	12[5]	10
Mozambique	Cliff, 1985a	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, Olamijulo & Joiner, 1982	104	97	3	3	1

Source: Galazka, 1993

1 Immunized during pregnancy with TT.

2 Immunized in childhood with DTP vaccine.

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

4 22 mothers "fully" immunized.

5 One mother received the second dose two days before delivery.

As with apparent TTCV failures reported in vaccinated children and adults, several explanations exist for reports of neonatal tetanus cases in infants of women claiming to be vaccinated:

1. *Inaccurate vaccination history*. Maternal vaccination status is often based on verbal history rather than on documentation. In many countries written records are not given to mothers, or they do not retain the records they are given. In some countries pregnant women receive many injections unrelated to tetanus immunization, and this may lead to confusion when documenting TTCV history. Discrepancies in the history of TT immunization were observed in three reports where the number of those immunized varied between documented evidence and verbal history compared to the levels of seropositive mothers (WHO, 1996a), and where 45% of women who said they had received no TT dose were considered seropositive (Scobie et al., 2016; Deming et al., 2002). This is likely to be due to residual immunity from infant doses and/or booster doses forgotten or unrecorded on vaccination cards, such as doses given during supplementary immunization activities.
2. *Receipt of vaccine late in pregnancy.* Many women report for antenatal care late in their pregnancy (see Section 6.2). Consequently, if immunization is required (see Section 10), the recommended number of doses is often completed too close to delivery (i.e. less than 2 weeks) to enable the mother to develop and transfer sufficient antitoxin for adequate newborn protection (although the vaccine will contribute to long-term immunity and will help to protect neonates in subsequent pregnancies).
3. *Low-potency vaccine*. The TT 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 TT. Hlady et al. (1992) reported no potency in three consecutive lots of vaccine produced locally in Bangladesh.
4. *Poor maternal immune response*. In most studies performed in developing countries, two doses of TT stimulated the development of tetanus antibody levels that were considered protective in 94% of women (Blencowe et al., 2010). Some mothers, however, may have an antibody response below the protective level due to immunological factors ("poor responders").
5. *Inadequate placental transfer*. Data are available to suggest that, 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 TTCV. It is important to note that the level of antibodies required to neutralize toxin is dependent on 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 tetanus toxin and the risks associated with practices leading to exposure are likely to differ by subpopulation.

8. Safety of tetanus toxoid

Tetanus toxoid is one of the most commonly used antigens in vaccine formulations worldwide. It is widely accepted as highly effective with an excellent safety profile. As with any vaccine, adverse events following immunization (AEFI) occur in some persons, though not all reported events are causally related to vaccine administration (WHO Vaccine Safety Basics). Numerous studies conducted in recent decades have investigated the safety of vaccination with TT. The most common adverse vaccine reactions (those AEFIs likely to have been caused by components of the vaccine) reported following administration of TT are mild, local reactions, including pain or tenderness at the injection site, oedema and erythema. Severe adverse events are extremely rare (Roper et al., 2013).

Multiple factors influence both whether an adverse event will occur following vaccination and the degree of severity of that event. Individual characteristics, including the number of previous doses of TTCVs received and the level of antitoxin antibodies at the time of vaccination, are positively associated with the likelihood and severity of adverse events. Vaccine formulation and administration characteristics – including the route of administration, the type and quantity of adjuvant, and the presence of other antigens in the vaccine formulation – have also been associated with adverse events (Roper et al., 2013). Evidence suggests that the time since previous administration of a TTCV may also increase the risk (Halperin et al., 2006; Jackson et al., 2009; Talbot et al., 2010).

Local reactions at the site of injection are the most common adverse event associated with TTCV 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, Cohen & Rose, 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 et al., 1973). More severe local reactions characterized by marked swelling are estimated to occur in fewer than 2% of vaccine recipients (Relihan, 1969; Sisk & Lewis, 1965). In addition, recent evaluation of combination of TTCV have found an acceptable safety profile in various age groups (Knutsson et al., 2001; Saenger et al., 2005; Huang et al., 2005; Mallet et al., 2004; Tapia et al., 2015; Vannice et al., 2015; Ouandaogo et al., 2012).

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 TTCV (Lloyd et al., 2003). In infants, fever and irritability are reported at higher rates, approximately 20–25% of them following administration of a TTCV (Knutsson et al., 2001; Mallet et al., 2004).

In 2014, the Institute of Medicine (IOM) of the USA evaluated the strength of the epidemiological and mechanistic evidence regarding the association between TT vaccines and numerous severe reactions and potentially life-threatening conditions (National Academies Press, 2014). The IOM concluded that the evidence was inadequate or non-existent to accept or reject that there is any causal relationship between TT vaccines and the following severe outcomes: acute disseminated encephalomyelitis (ADEM), arthropathy, ataxia, autism, Bell's palsy, chronic inflammatory disseminated polyneuropathy (CIDP), chronic uticaria, encephalitis, encephalopathy, fibromyalgia, immune thrombocytopenic purpura (ITP), infantile spasms, Guillain-Barre syndrome (GBS), multiple sclerosis in adults, relapse of multiple sclerosis in adults, relapse of multiple sclerosis in children, myocarditis, opsoclonus myoclonus syndrome (OMS), optic neuritis, seizures, serum sickness, sudden infant death syndrome (SIDS) and transverse myelitis. In addition, the IOM report rejected a causal relationship between TT vaccine and type I diabetes on the basis of evidence from previous studies (National Academies Press, 2014).

However, the IOM assessment found that "the evidence convincingly supports a causal relationship between tetanus toxoid vaccine and anaphylaxis" (National Academies Press, 2014). Anaphylactic reactions to tetanus toxoid are rare (Wassilak et al., 2004; National Academies Press, 2014; 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). However, one study used skin-testing to show that children who had experienced an allergic reaction following TT vaccine reacted to the TT rather than to excipients (aluminium phosphate or thiomersal) (Mayorga et al., 2003), though the sensitivity of skin-testing has been questioned (Roper et al., 2017). A records review based on passive surveillance of anaphylaxis associated with childhood vaccines (Bohlke et al., 2003) found four cases of anaphylaxis associated with TT-containing vaccines (DT, DTP, DTaP, DTP-Hib, or Td) in approximately 2 million doses administered, though the authors note that the vaccines were often administered in combination with other vaccines. Between the initiation of vaccine adverse event reporting to the CDC in 1978 and 1996, no deaths caused by anaphylaxis were reported although more than 80 million doses of DTP were administered during that time (ACIP, 1996). An analysis of reports made to the US National Vaccine Injury Compensation Program between 2000 and 2009 included one case of anaphylaxis in an infant (following DTaP) and one in an older adult (following Td) resulting in death (Johann-Liang, Josephs & Dreskin, 2011; Miller et al., 2015).

TT is safe for pregnant women, and vaccination during pregnancy is recommended (WHO, 2017a; WHO, 2017b). Evidence suggests that there is no increased risk of adverse events among pregnant women and no increased risk of adverse birth outcomes for women who receive Tdap during pregnancy compared to those who do not, regardless of whether they have been vaccinated with TTCV <2 years, 2–5 years or more than 5 years previously (Morgan et al., 2015; Sukumaran et al., 2015). These studies did not suggest any increased risk of adverse events even if vaccination occurred both soon before pregnancy and during pregnancy, contrary to other evidence that suggests that a short duration between tetanus toxoid doses is associated with increased risk of adverse events in non-pregnant individuals.

Immunization of women during pregnancy has been shown to be a safe and effective approach to reducing maternal and neonatal tetanus and a key strategy for its elimination.

9. Combination vaccines and concomitant vaccine use

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

The Hib conjugate comprises Hib-polyribosylribitol phosphate (PRP) covalently linked to TT. Studies have shown that maternal antibodies to tetanus antitoxin do not interfere with the response to Hib conjugate vaccines but may enhance responses (Kurikka et al., 1996; Nohynek et al., 1999; Voysey et al., 2017; Ladhani et al., 2015) (see Section 6.5) and that simultaneous administration of DTP and Hib conjugate does not increase the frequency of common adverse events (Holmes et al., 1993). However, Hib conjugate vaccines cannot replace the need for primary TT immunization (Carlsson et al., 1994).

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 conjugate vaccines has raised concerns about impairment of or interference with responses, plus other safety concerns, but there is also the potential to enhance responses to the combination of antigens administered (Kitchin et al., 2007).

Significant enhancement of the tetanus response has been observed in infants in the United Kingdom following concomitant administration of diphtheria, tetanus and whole-cell pertussis with Hib conjugate and a meningococcal serogroup C TT conjugate (Table 5 and Kitchin et al., 2007). Augmentation of the tetanus response has also been observed when a five-component acellular pertussis vaccine is used in place of whole-cell pertussis vaccine (Kitchin et al., 2007).

Table 5: Effect of concomitant meningococcal serogroup C conjugate (MCC-TT) vaccine on response to *Haemophilus influenzae* type b (Hib) and tetanus in United Kingdom infants (2–3–4-month schedule) receiving DTwP/Hib

Vaccines given	PRP GMC ug/mL	Tetanus GMC IU/mL
DTwP + PRP-T*[7]	4.50 (3.21–6.32)	0.65 (0.54–0.77)
DTwP/PRP-T*	3.39 (2.48–4.66)	1.19 (0.98–1.44)
DTwP/PRP-T+MCC-TT **[8] (10Lfs in DTP + 30ug PRP-T+15ug MCC-T)	11.59 (9.3–14.5)	4.00 (3.3–4.8)

*Sources: * Begg et al., 1995; ** Richmond et al., 2001.*

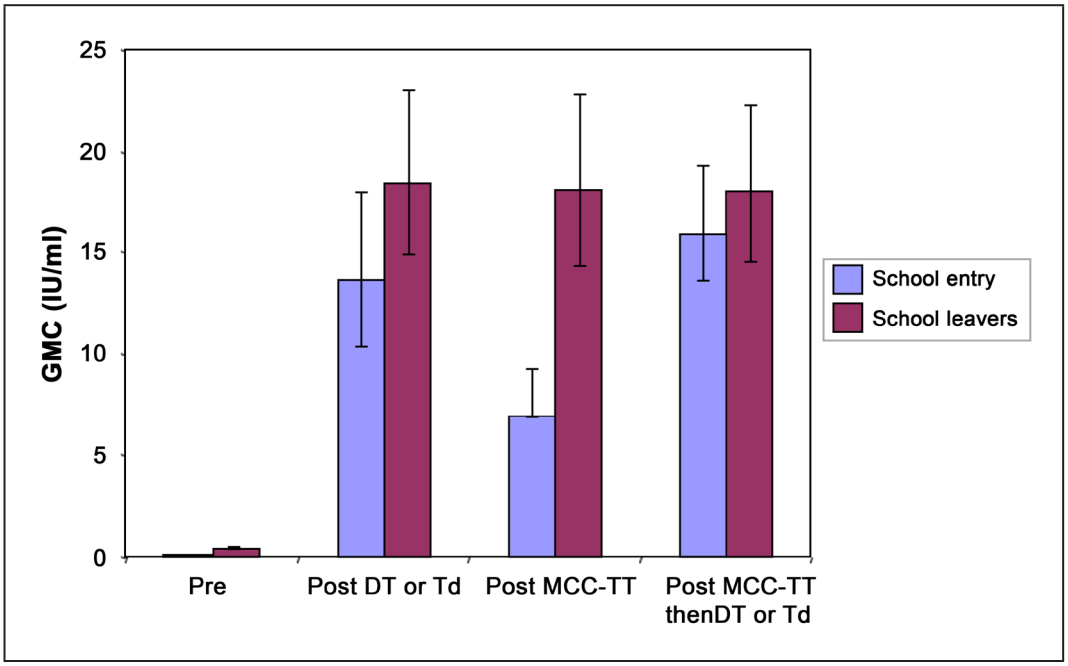
[7] PRP = polyribosylribitol phosphate;

[8] MCC = meningococcal serogroup C conjugate.

Data from the United Kingdom demonstrated the potential for TT-conjugate vaccines containing TT as the carrier protein to boost antibody responses to tetanus (Burrage et al., 2002). Children of school-entry age (3.5–6 years) and school leavers (13–18 years) were found to make a substantial tetanus antibody response (geometric mean concentration [GMC] > 5 IU/mL) to a meningococcal serogroup C (MCC) TT-conjugate vaccine (Figure 7). In school leavers, the tetanus antibody level was comparable following administration of either the MCC-TT or a Td vaccine (Figure 7). 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 10 months of age (Nohynek et al., 1999). This brings into question whether the response to TT used as a carrier protein in conjugate vaccines is sufficient or not to allow replacement of the routine boosting dose. However, the number of new combination vaccines provided in countries using the EPI schedule may be limited, mainly due to cost.

A relevant development was the introduction of a meningococcal serogroup A conjugate vaccine (MenAfriVac) that is conjugated to TT and has been delivered in campaigns targeting ages 1–29 years in Africa's "meningitis belt" (Aguado et al., 2015). The clinical trials of MenAfriVac demonstrated that robust tetanus serological responses were generated in 1–29-year-olds and were similar to those expected after a booster dose of TT (Figure 8) (Borrow et al., 2015). Increases in tetanus toxoid antibody levels were also demonstrated in a serosurvey in Mali performed before (2010) and after (2012) the introduction of MenAfriVac (Basta et al., 2015). A total of 793 pre-campaign and 800 post-campaign sera were tested and the percentage of subjects with antibody levels ≥ 0.1 IU/mL increased from 57% to 88%. The high acceptance of the MenAfriVac immunization campaigns meant that a large fraction of women of reproductive age received MenAfriVac. In a review of incidence data for neonatal tetanus for countries with and without MenAfriVac campaigns in 1–29-year-olds, it was found that cases of tetanus fell by 25% in countries that completed MenAfriVac campaigns (Borrow et al., 2015). In addition, these campaigns have the potential to close tetanus immunity gaps in older children and adult men, as was demonstrated in Mali's pre- and post-campaign serosurvey (Basta et al., 2015).

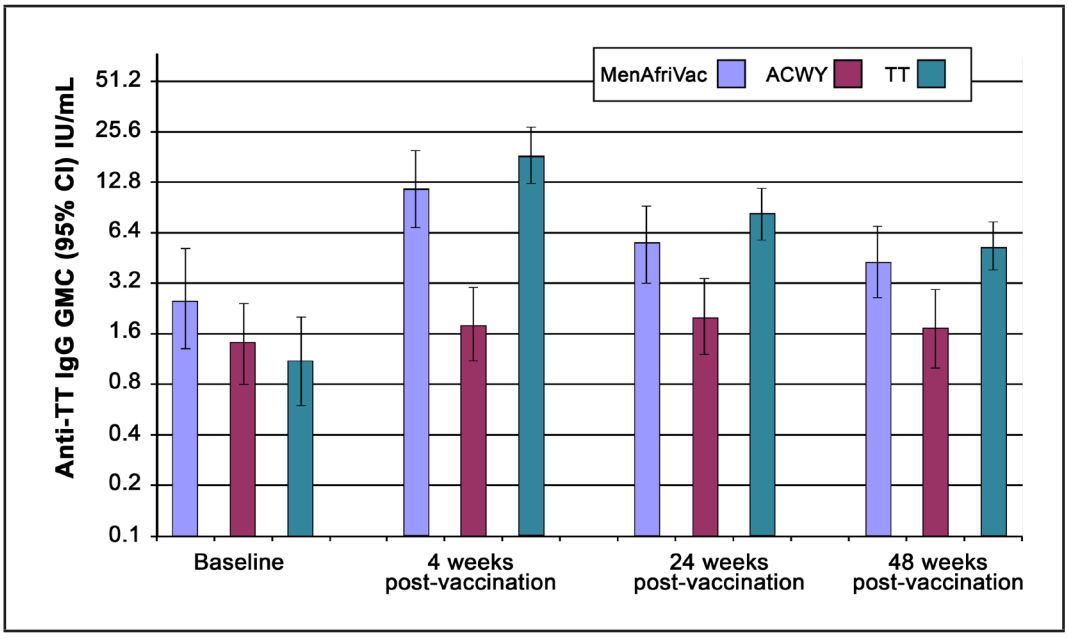
Figure 7: Effect of meningococcal TT conjugates on the tetanus antibody levels induced following diphtheria-tetanus vaccines given at school entry or to school leavers



Source: Burrage et al., 2002.
Reprint of an Open Access article distributed under the terms of the Creative Commons Attribution License

GMC = geometric mean concentration.

Figure 8: Anti-TT IgG geometric mean concentrations (95% CI) for healthy adults aged 18–35 years following vaccination with meningococcal A conjugate vaccine, meningococcal ACWY polysaccharide vaccine or tetanus toxoid vaccine



Source: Borrow et al., 2015.
Reprint, permission obtained by publisher.

Although tetanus conjugated vaccines generally boost immunity, some studies have shown the converse. 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 in terms of inducing protective levels of antitoxin. Carrier-induced epitope-specific suppression is a phenomenon whereby the antibody response to a specific antigen is inhibited following prior immunization with the carrier protein. It is thought that this is due to the expansion of carrier-specific B cells (Barington 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.

10. Implications for immunization programmes

In 2016 WHO updated its position paper on tetanus vaccines (WHO, 2017a). In addition to the continuing challenge of achieving global MNTE, the recent cases of tetanus in men and adolescent boys undergoing voluntary male medical circumcision (VMMC) to reduce HIV transmission highlighted the immunity gap in men in low-income countries — the legacy of poor infant immunization coverage and a lack of subsequent boosters to maintain tetanus immunity throughout the life-course (Dalal et al., 2016).

In 1998 WHO recommended that the use of tetanus toxoid (TT) vaccine should be replaced with tetanus-diphtheria (Td) vaccine following large outbreaks of diphtheria in the Soviet Union and other countries despite the high coverage of routine childhood vaccination. The recommendation to shift from using TT to Td vaccine was re-stated by SAGE in 2002 and 2016, and was published and disseminated again in the WHO position papers on tetanus vaccine in 2006 and 2017 (WHO 2006 and 2017a). The updated WHO position paper recommends that, in addition to the three primary TTCV doses in the first year of life, three additional booster doses should be given at 12–23 months, 4–7 years and 9–15 years with ideally at least 4 years between booster doses. This schedule is designed to maintain immunity throughout adult life, both in women of reproductive age to achieve MNTE and also to ensure equitable protection of all persons as part of broader tetanus control. Although immunogenicity data suggest that tetanus antitoxin levels after three primary doses in the first year of life will remain above the protective threshold until 3–4 years of age in many children without a booster in the second year of life (see Section 5, Figure 3), the WHO recommendation is designed to maximize coverage of the first booster dose, as well as providing an early opportunity to complete primary immunization if TT doses in the first year have been missed. More generally it reflects the need to develop a platform for administering vaccines in the second year of life in settings where the EPI schedule provides the only opportunity to receive vaccinations in childhood (Sodha & Dietz, 2015).

The target for elimination of maternal and neonatal tetanus requires the number of neonatal deaths due to tetanus to be less than 1 per 1000 live births in every district. Much success has been achieved in the progress towards this goal since the World Health Assembly first called for global neonatal tetanus elimination in 1989 (World Health Assembly, 1989). Because of slow implementation of the recommended MNTE strategies, the target date for the attainment of elimination by all countries was postponed to 2000. In 1999, when progress towards the attainment of the global elimination goal was reviewed, the target date was reset to 2005 which was later shifted to 2015. By the end of 2015, there were still 21 countries that still had not attained elimination; this number was down to 18 countries by the end of 2016, and 16 countries by the end of June 2017 (WHO, 2017b).

The global MNTE Initiative promotes a multifaceted approach, incorporating immunization of women of reproductive age with TTCVs, 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 reproductive age with the goal of providing protection throughout reproductive years (WHO 1999a; WHO, 2002). Data have confirmed the impact of immunizing all women of childbearing years on the incidence of neonatal tetanus (Hamid et al., 1985; Rahman et al., 1982; WHO, 1999b; Koenig et al., 1998; WHO, 1996c) (see Section 7).

An important strategy in MNTE has been the "high-risk" approach (WHO, 1999a; WHO, 2002) which uses supplementary immunization campaigns for women of reproductive 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 rapidly increases population immunity among the current cohort of women of reproductive age. It is essential to strengthen immunization programmes at the same time in order to ensure that MNTE is sustained in the long term. Improvements in other areas of health care are recognized as important factors in eliminating neonatal tetanus.

Until the susceptibility gap in males in low-income countries has been closed by the introduction of routine boosters in childhood and adolescence in order to minimize risks of tetanus associated with VMMC, WHO has recommended a dual protection approach (WHO, 2017c), namely: 1) clean care, including enhanced attention to standard protocols for skin preparation and cleanliness both at the facility and by individuals who undergo the circumcision procedure, irrespective of the method of VMMC, and 2) ensuring that all clients are adequately protected against tetanus by vaccination before circumcision. Where circumcision is performed with a device method that leaves the foreskin in situ to be removed several days after application, the procedure should be undertaken only if there is evidence of adequate protection against tetanus by immunization with TTCV. This includes: two TTCV doses at least 4 weeks apart, with the second dose at least 2 weeks before device placement, or – if a client has previously received three infant doses, or one dose during adolescence or adulthood – a TTCV booster at least 2 weeks before device placement (a booster at the time of placement provides only limited protection because it takes 7–14 days for antibodies to rise to protective levels) or a series of five doses of TTCV.

References

1. **Abrahamian FM, Pollack CV Jr, LoVecchio F, Nanda R, Carlson RW (2000).** Fatal tetanus in a drug abuser with "protective" anti-tetanus antibodies. *J Emerg Med. 18:189–93.*

2. **ACIP (Immunization Practices Advisory Committee, USA) (1996).** Update: Vaccine side-effects, adverse reactions, contraindications and precautions. *MMWR. CDC surveillance summaries: 45:1–35.*

3. **Aggerbeck H, Norgaard-Pedersen B, Heron I (1996).** Simultaneous quantitation of diphtheria and tetanus antibodies by double antigen, time-resolved fluorescence immunoassay. *J Immunol Methods. 190:171–83.*

4. **Aguado MT, Jodar L, Granoff D, Rabinovich R, Ceccarini C, Perkin GW (2015).** From epidemic meningitis vaccines for Africa to the Meningitis Vaccine Project. *Clin Infect Dis. 61(Suppl 5):S391–5.*

5. **Amanna IJ, Carlson NE, Slifka MK (2007).** Duration of humoral immunity to common viral and vaccine antigens. *N Engl J Med. 357:1903–15.*

6. **Amstey MS, Insel R, Munoz J, Pichichero M (1985).** Fetal-neonatal passive immunization against Hemophilus influenzae type b. *Am J Obstet Gynecol. 153:607–11.*

7. **Baker CJ1, Rench MA, Edwards MS, Carpenter RJ, Hays BM, Kasper DL (1988).** Immunization of pregnant women with a polysaccharide vaccine of group B *Streptococcus*. *N Engl J Med. 319:1180–5.*

8. **Barhate G, Gautam M, Gairola S, Jadhav S, Pokharkar V (2014).** Enhanced mucosal immune responses against tetanus toxoid using novel delivery system comprised of chitosan-functionalized gold nanoparticles and botanical adjuvant: characterization, immunogenicity, and stability assessment. *J Pharm Sci. 103:3448–56.*

9. **Barington T, Skettrup M, Juul L, Heilmann C (1993).** Non-epitope-specific suppression of the antibody response to *Haemophilus influenzae* type b conjugate vaccines by pre-immunization with vaccine components. I*nfect Immun. 61:432–8.*

10. **Basta NE, Borrow R, Berthe A, Onwuchekwa U, Dembélé AT, Almond R et al. (2015).** Higher tetanus toxoid immunity 2 years after PsA-TT introduction in Mali. *Clin Infect Dis. 61(Suppl 5):S578–85.*

11. **Begg NT, Miller E, Fairley CK, Chapel HM, Griffiths H, Waight PA et al. (1995).** Antibody responses and symptom s after DTP and either tetanus or diphtheria *Haemophilus influenzae* type B conjugate vaccines given for primary immunization by separate or mixed injection. *Vaccine. 13:1547–50.*

12. **Beltran A, Go E, Haq M, Clarke HB, Zaman M, Recco RA (2007).** A case of clinical tetanus in a patient with protective antitetanus antibody level. *South Med J. 100:83.*

13. **Berggren GG, Berggren W, Verly A, Garnier N, Peterson W, Ewbank D et al. (1983).** Traditional midwives, tetanus immunization and infant mortality in rural Haiti. *Trop Doct. 13:79–87.*

14. **Bildhaiya GS (1983).** A study of tetanus in a hospital at Ahmedabad. *J Indian Med Assoc. 80:21–3.*

15. **Black RE, Huber D H, 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. *Bull World Health Organ. 58:927–30.*

16. **Blencowe H, Lawn J, Vandelaer J, Roper M, Cousens S (2010).** Tetanus toxoid immunization to reduce mortality from neonatal tetanus. *Int J Epidemiol. 39 (Suppl 1):i102–9.*

17. **Bohlke K, Davis RL, Marcy SM, Braun MM, DeStefano F, Black SB et al.; Vaccine Safety Datalink Team (2003).** Risk of anaphylaxis after vaccination of children and adolescents. *Pediatrics. 112:815–20.*

18. **Booy R, Aitken SJ, Taylor S, Tudor-Williams G, Macfarlane JA, Moxon ER et al. (1992).** Immunogenicity of combined diphtheria, tetanus, and pertussis vaccine given at 2, 3, and 4 months versus 3, 5, and 9 months of age. *Lancet. 339:507–10.*

19. **Borkowsky W, Rigaud M, Krasinski K, Moore T, Lawrence R, Pollack H (1992).** Cell-mediated and humoral immune responses in children infected with human immunodeficiency virus during the first four years of life. *J Pediatr. 120:371–5.*

20. **Borrow R, Miller E (2006).** Surrogates of protection. *In: Frosch M, Maiden M, editors.*Handbook of meningococcal disease. *Hoboken (NJ): Wiley:323–51.*

21. **Borrow R, Tang Y, Yakubu A, Kulkarni PS, LaForce FM (2015).** MenAfriVac as an antitetanus vaccine. *Clin Infect Dis. 61(Suppl 5):S570–7.*

22. **Böttiger M, Gustavsson O, Svensson A. (1998).** Immunity to tetanus, diphtheria and poliomyelitis in the adult population of Sweden in 1991. *International Journal of Epidemiology. 27:916-25.*

23. **Brabin BJ, Nagel J, Hagenaars AM, Ruitenberg E, van Tilborgh AM (1984).** The influence of malaria and gestation on the immune response to one and two doses of adsorbed tetanus toxoid in pregnancy. *Bull World Health Organ. 62:919–30.*

24. **Bracebridge S, Crowcroft N, White J (2004).** Tetanus immunisation policy in England and Wales – an overview of the literature. *Commun Dis Public Health. 7:283–6.*

25. **Brair ME, Brabin BJ, Milligan P, Maxwell S, Hart CA (1994).** Reduced transfer of tetanus antibodies with placental malaria. *Lancet. 343:208–9.*

26. **Breman JG, Wright GG, Levine L, Latham WC, Compaoré KP (1981).** The primary serological response to a single dose of adsorbed tetanus toxoid, high concentration type. *Bull World Health Organ. 59:745–52.*

27. **Burrage M, Robinson A, Borrow R, Andrews N, Southern J, Findlow J 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. *Infect Immun. 70:4946–54.*

28. **Caglar K, Karakus R, Aybay C (2005).** Determination of tetanus antibodies by a double-antigen enzyme-linked immunosorbent assay in individuals of various age groups. *Eur J Clin Microbiol Infect Dis. 24:523–8.*

29. **Carlsson RM, Claesson BA, Iwarson S, Lagergård T, Käyhty H (1994).** Antibodies against *Haemophilus influenzae* type b and tetanus in infants after subcutaneous vaccination with PRP-T / diphtheria or PRP- OMP/ diphtheria-tetanus vaccines. *Pediatr Infect Dis J. 13:27–33.*

30. **CDC (2011).** Tetanus surveillance – United States, 2001–2008. Atlanta (GA): Centers for Disease Control and Prevention. *MMWR. 60(12):365–9* (*https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6012a1.htm*, accessed 11 August 2018).

31. **CDC (2018a).** Recommended immunization schedule for children and adolescents aged 18 years or younger, United States. Atlanta (GA): Centers for Disease Control and Prevention (*https://www.cdc.gov/vaccines/schedules/hcp/imz/child-adolescent.html*, accessed 11 August 2018).

32. **CDC (2018b).** Recommended immunization schedule for adults aged 19 years or older, United States,. Atlanta (GA): Centers for Disease Control and Prevention (*https://www.cdc.gov/vaccines/schedules/hcp/imz/adult.html*, accessed 11 August 2018).

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. *Indian J Med Res. 52:1241–9.*

34. **Cliff J (1985a).** Neonatal tetanus in Maputo, Mozambique. I. Hospital incidence and childbirth practices. *Cent Afr J Med. 31:9–12.*

35. **Cliff J (1985b).** Neonatal tetanus in Maputo, Mozambique. II. Preventive measures. *Cent Afr J Med. 31:27–32.*

36. **Colombet I, Saguez C, Sanson-Le Pors MJ, Coudert B, Chatellier G, Espinoza P; Scientific Committee of the TetaQuick 1000 Study (2005).** Diagnosis of tetanus immunization status: multicenter assessment of a rapid biological test. *Clin Diagn Lab Immunol. 12:1057–62.*

37. **Cowles PB (1937).** Tetanus immunization. *Yale J Biol Med. 9:409–416.*

38. **Cox JC, Premier RR, Finger W, Hurrell JG (1983).** A comparison of enzyme immunoassay and bioassay for the quantitative determination of antibodies to tetanus toxin. *J Biol Stand. 11:123–8.*

39. **Crone NE, Reder AT (1992).** Severe tetanus in immunized patients with high anti-tetanus titers. *Neurology. 42:761–4.*

40. **Cumberland P, Shulman CE, Maple PA, Bulmer JN, Dorman EK, Kawuondo K et al. (2007).** Maternal HIV infection and placental malaria reduce transplacental antibody transfer and tetanus antibody levels in newborns in Kenya. *J Infect Dis. 196:550–7.*

41. **Dagan R, Goldblatt D, Maleckar JR, Yaïch M, Eskola J (2004).** Reduction of antibody response to an 11-valent pneumococcal vaccine coadministered with a vaccine containing acellular pertussis components. *Infect Immun. 72:5383–91.*

42. **Dalal S, Samuelson J, Reed J, Yakubu A, Ncube B, Baggaley R (2016).** Tetanus disease and deaths in men reveal need for vaccination. *Bull World Health Organ. 94:613–21.*

43. **Danilova E, Shiryayev A, Kristoffersen EK, Sjursen H (2005).** Attenuated immune response to tetanus toxoid in young healthy men protected against tetanus. *Vaccine. 23:4980–3.*

44. **Dastur F, Awatramani V, Dixit JA (1981).** Response to single dose of tetanus vaccine in subjects with naturally acquired tetanus antitoxin. *Lancet. 2:219–21.*

45. **Deivanayagam N, Nedunchelian K, Kamala KG (1991).** Neonatal tetanus: observations on antenatal immunization, natal and immediate post-natal factors. *Indian J Pediatr. 58:119–22.*

46. **de Melker HE, van den Hof S, Berbers GA, Nagelkerke NJ, Rümke HC, Conyn-van Spaendonck MA (2000).** A population-based study on tetanus antitoxin levels in the Netherlands. *Vaccine. 18:100–8.*

47. **Demicheli V, Barale A, Rivetti A (2015).** Vaccines for women for preventing neonatal tetanus. *Cochrane Database Syst Rev. 7: Cd002959.*

48. **Deming MS, Roungou JB, Kristiansen M, Heron I, Yango A, Guenengafo A et al. (2002).** Tetanus toxoid coverage as an indicator of serological protection against neonatal tetanus. *Bull World Health Organ. 80:696–703.*

49. **de Moraes-Pinto MI, Oruamabo RS, Igbagiri FP, Chan MC, Prado SM, Vancetto MD et al. (1995).** Neonatal tetanus despite immunization and protective antitoxin antibody. *J Infect Dis. 171:1076–7.*

50. **de Moraes-Pinto MI, Almeida AC, Kenj G, Filgueiras TE, Tobias W, Santos AM et al. (1996).** Placental transfer and maternally acquired neonatal IgG immunity in human immunodeficiency virus infection. *J Infect Dis. 173:1077–84.*

51. **de Moraes-Pinto MI, Verhoeff F, Chimsuku L, Milligan PJ, Wesumperuma L, Broadhead RL et al. (1998).** Placental antibody transfer: influence of maternal HIV infection and placental malaria. *Arch Dis Child Fetal Neonatal Ed. 79:F202–5.*

52. **de Voer RM, van der Klis FR, Nooitgedagt JE, Versteegh FG, van Huisseling JC, van Rooijen DM et al. (2009).** Seroprevalence and placental transportation of maternal antibodies specific for *Neisseria meningitidis* serogroup C, *Haemophilus influenzae* type B, diphtheria, tetanus, and pertussis. *Clin Infect Dis. 49:58–64.*

53. **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. *Indian J Med Res. 63:583–9.*

54. **Dietz V, Galazka A, van Loon F, Cochi S (1997).** Factors affecting the immunogenicity and potency of tetanus toxoid: implications for the elimination of neonatal and non-neonatal tetanus as public health problems. *Bull World Health Organ. 75(1):81–93.*

55. **Dokmetjian J, Della Valle C, Lavigne V, de Luján CM, Manghi MA (2000).** A possible explanation for the discrepancy between ELISA and neutralizing antibodies to tetanus toxin. V*accine. 18:2698–703.*

56. **Edsall G, Elliott MW, Peebles TC, Eldred MC (1967).** Excessive use of tetanus toxoid boosters. *JAMA. 202:17–9.*

57. **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. L*yon: Fondation Merieux:683–5.*

58. **Edsall G (1976).** Problems in the immunology and control of tetanus. *Med J Aust. 2:216–20.*

59. **Einterz EM, Bates ME (1991).** Caring for neonatal tetanus patients in a rural primary care setting in Nigeria: a review of 237 cases. *J Trop Pediatr. 37:179–81.*

60. **Eisen AH, Cohen JJ, Rose B (1963).** Reaction to tetanus toxoid: a report of a case with immunologic studies. *N Engl J Med. 269:1408–11.*

61. **Elkharrat D, Sanson-Le-Pors MJ, Arrouy L, Beauchet A, Benhamou F (2010).** Evaluation of a bedside immunotest to predict individual anti-tetanus seroprotection: a prospective concordance study of 1018 adults in an emergency department. *Emerg Med J. 27:36–42.*

62. **El-Sherbini A (1991).** Study of tetanus neonatorum in Tanta Fever Hospital, 1988–1989. *J Trop Pediatr. 37:262–3.*

63. **Embree J, Law B, Voloshen T2, Tomovici A (2015).** Immunogenicity, safety, and antibody persistence at 3, 5, and 10 years postvaccination in adolescents randomized to booster immunization with a combined tetanus, diphtheria, 5-component acellular pertussis, and inactivated poliomyelitis vaccine administered with a hepatitis B virus vaccine concurrently or 1 month apart. *Clin Vaccine Immunol. 22:282–90.*

64. **Englund JA (2007).** The influence of maternal immunization on infant immune responses. *J Comp Pathol. 137(Suppl 1):S16–9.*

65. **Ergönül O, Sözen T, Tekeli E (2001).** Immunity to tetanus among adults in Turkey. *Scand J Infect Dis. 33:728–30.*

66. **ECDC (2015).** Tetanus – annual epidemiological report for 2015. Solna: European Centre for Disease Prevention and Control (*https://ecdc.europa.eu/en/publications-data/tetanus-annual-epidemiological-report-2015*, accessed 11 August 2018).

67. **Evans DG (1943).** Persistence of antitoxin in man following active immunization. *Lancet. 2:316–7.*

68. **Gad ZM, Sherif AA, Hasab AA, Hassan MN (1986).** An epidemiological study on tetanus neonatorum in Alexandria. *Bulletin of the High Institute of Public Health. 16:33-45.*

69. **Galazka AM.** The immunological basis for immunization series (1993). Module 3: Tetanus. Geneva: *World Health Organization (Document No. WHO/EPI/Gen93.13).*

70. **Galazka A, Milstien J, Zaffran M (1998).** Thermostability of vaccines. Geneva: *World Health Organization* (*http://apps.who.int/iris/bitstream/10665/64980/1/WHO_GPV_98.07.pdf*, accessed 11 August 2018).

71. **Gall SA, Myers J, Pichichero M (2011).** Maternal immunization with tetanus-diphtheria-pertussis vaccine: effect on maternal and neonatal serum antibody levels. *Am J Obstet Gynecol. 204:334.e1–5.*

72. **Gendrel D, Richard-Lenoble D, Massamba MB, Picaud A, Francoual C, Blot P (1990b).** Placental transfer of tetanus antibodies and protection of the newborn. *J Trop Pediatr. 36:279–82.*

73. **Ghosh JB (1990).** Prevention of tetanus neonatorum. *Indian Pediatr. 27:210.*

74. **Gidding HF, Backhouse JL, Burgess MA, Gilbert GL (2005).** Immunity to diphtheria and tetanus in Australia: a national serosurvey. *Medical J Aust. 183:301–4.*

75. **Gilles HM, Greenwood BM, Greenwood AM, Bradley AK, Blakebrough I, Pugh RN et al. (1983).** The Malumfashi project – an epidemiological, clinical and laboratory study. *Trans R Soc Trop Med Hyg. 77:24–31.*

76. **Gold H (1937).** Studies on tetanus toxoid. I. Active immunization of allergic individuals with tetanus toxoid, alum precipitated, refined. *J Allergy. 8:230–45.*

77. **Goulon M, Girard O, Grosbuis S, Desormeau JP, Capponi MF (1972).** Les anticorps antitetaniques. Titrage avant seroanatoxiono-therapie chez 64 tetaniques [Antitetanus antibodies. Assay before anatoxinotherapy in 64 tetanus patients]. *La Nouvelle Presse Médicale. 1:3049–50.*

78. **Grange AO (1991).** Neonatal tetanus in Lagos metropolis. *Niger J Paediatr. 18:12–21.*

79. **Greenwood BM, Bradley-Moore AM, Bryceson AD, Palit A (1972).** Immunosuppression in children with malaria. *Lancet. 1:169–72.*

80. **Grimprel E, Wysocki J, Boisnard F, Thomas S, Mwawasi G, Reynolds D (2011).** Immunogenicity and safety of fully liquid $DTaP_5$-IPV-Hib compared with $DTaP_3$-IPV/Hib when both coadministered with a heptavalent pneumococcal conjugate vaccine (PCV7) at 2, 3, 4, and 12 to 18 months of age: a phase III, single-blind, randomised, controlled, multicentre study. *Vaccine. 29:7370–8.*

81. **Grudeborn B (1987).** 199 casos de tetano neonatal em Luanda [199 cases of neonatal tetanus in Luanda]. *Acta Medica Angola, 6:17–33.*

82. **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. *J Biol Stand. 12:145–9.*

83. **Gupta RK, Maheshwari SC, Singh H (1985).** The titration of tetanus antitoxin. IV. Studies on the sensitivity and reproducibility of the toxin neutralization test. *J Biol Stand. 13:143–9.*

84. **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. 22:215–9.*

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

86. **Halperin SA, Sweet L, Baxendale D, Neatby A, Rykers P, Smith B et al. (2006).** How soon after a prior tetanus-diphtheria vaccination can one give adult formulation tetanus-diphtheria acellular pertussis vaccine? *Pediatr Infect Dis J. 25:195–200.*

87. **Hamid ED, Daulay AP, Lubis CP, Rusdidjas, Siregar H (1985).** Tetanus neonatorum in babies delivered by traditional birth attendants in Medan, Indonesia. *Paediatrica Indonesiana. 25:167–74.*

88. **Hammarlund E, Thomas A, Poore EA, Amanna IJ, Rynko AE, Mori M et al. (2016).** Durability of vaccine-induced immunity against tetanus and diphtheria toxins: a cross-sectional analysis. *Clin Infect Dis. 62:1111–8.*

89. **Hardegree MC, Barile MF, Pittman M, Schofield FD, MacLennan R, Kelly A (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. *Bull World Health Organ. 43:439–51.*

90. **Hatamabadi HR, Abdalvand A, Safari S, Kariman H, Dolatabadi AA, Shahrami A et al. (2011).** Tetanus Quick Stick as an applicable and cost-effective test in assessment of immunity status. *Am J Emerg Med. 29v717–20.*

91. **Hendriksen CF, vd Gun JW, Nagel J, Kreeftenberg JG (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. *J Biol Stand. 16:287–97.*

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

93. **Hlady WG, Bennett JV, Samadi AR, Begum J, Hafez A, Tarafdar AI et al. (1992).** Neonatal tetanus in rural Bangladesh: risk factors and toxoid efficacy. *Am J Public Health. 82:1365–9.*

94. **Holmes SJ, Fritzell B, Guito KP, Esbenshade JF, Blatter MM, Reisinger KS et al. (1993).** Immunogenicity of *Haemophilus influenzae* type b polysaccharide-tetanus toxoid conjugate vaccine in infants. *Am J Dis Child. 147:832–6.*

95. **Hong HA, Ke NT, Nhon TN, Thinh ND, van der Gun JW, Hendriks JT, et al. (1996).** Validation of the combined toxin-binding inhibition test for determination of neutralizing antibodies against tetanus and diphtheria toxins in a vaccine field study in Viet Nam. *Bulletin World Health Organization. 74:275-82.*

96. **Huang LM, Chang LY, Tang H, Bock HL, Lu CY, Huang FY et al. (2005).** Immunogenicity and reactogenicity of a reduced-antigen-content diphtheria-tetanus-acellular pertussis vaccine in healthy Taiwanese children and adolescents. *J Adolesc Health. 37:517.e1–5.*

97. **Jackson LA, Yu O, Belongia EA, Hambidge SJ, Nelson J, Baxter R et al. (2009).** Frequency of medically attended adverse events following tetanus and diphtheria toxoid vaccine in adolescents and young adults: a Vaccine Safety Datalink study. *BMC Infect Dis. 9:165.*

98. **Jódar L1, Butler J, Carlone G, Dagan R, Goldblatt D, Käyhty H et al. (2003).** Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. *Vaccine. 21:3265–72.*

99. **Johann-Liang R, Josephs S, Dreskin SC (2011).** Analysis of anaphylaxis cases after vaccination: 10-year review from the National Vaccine Injury Compensation Program. *Ann Allergy Asthma Immunol. 106:440–3.*

100. **Kitchin NR, Southern J, Morris R, Hemme F, Thomas S, Watson MW et al. (2007).** Evaluation of a diphtheria-tetanus-acellular pertussis-inactivated poliovirus-Haemophilus influenzae type b vaccine given concurrently with meningococcal group C conjugate vaccine at 2, 3 and 4 months of age. *Arch Dis Child. 92:11–6.*

101. **Knutsson N, Trollfors B, Taranger J, Bergfors E, Sundh V, Lagergård T 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. 19:4396–403.*

102. **Koenig MA, Roy NC, McElrath T, Shahidullah M, Wojtyniak B (1998).** Duration of protective immunity conferred by maternal tetanus toxoid immunization: further evidence from Matlab, Bangladesh. *Am J Public Health. 88:903–7.*

103. **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. 105:843–53.*

104. **Kumar V, Kumar R, Mathur VN, Raina N, Bhasin M, Chakravarty A (1988).** Neonatal tetanus mortality in a rural community of Haryana. *Indian Pediatr. 25:167–9.*

105. **Kurikka S, Olander RM, Eskola J, Käyhty H (1996).** Passively acquired anti-tetanus and anti- *Haemophilus* antibodies and the response to *Haemophilus influenzae* type b-tetanus toxoid conjugate vaccine in infancy. *Pediatr Infect Dis J. 15:530–5.*

106. **Kurtzhals JA, Kjeldsen K, Hey AS, Okong'o-Odera EA, Heron I (1997).** Immunity to tetanus and diphtheria in rural Africa. *Am J Trop Med Hyg. 56:576–9.*

107. **Ladhani SN, Andrews NJ, Southern J, Jones CE, Amirthalingam G, Waight PA et al. (2015).** Antibody responses after primary immunization in infants born to women receiving a pertussis-containing vaccine during pregnancy: single arm observational study with a historical comparator. *Clin Infect Dis. 61:1637–44.*

108. **Lahiri DC (1939).** Absence of specific antitoxin in persons exposed to risk of tetanus infection. *Indian J Med Res. 27:581–3.*

109. **Launay O, Toneatti C, Bernède C, Njamkepo E, Petitprez K, Leblond A (2009).** Antibodies to tetanus, diphtheria and pertussis among healthy adults vaccinated according to the French vaccination recommendations. *Hum Vaccin. 5:341–6.*

110. **Layton GT. (1980).** A micro-enzyme-linked immunosorbent assay (ELISA) and radioimmunosorbent technique (RIST) for the detection of immunity to clinical tetanus. *Med Lab Sci. 37:323-9.*

111. **Levine L, Wyman L (1964).** A nationwide serum survey of United States military recruits, 1962. V. Serologic immunity to tetanus. *Am J Hyg. 80:314–9.*

112. **Levine L, McComb JA, Dwyer RC, Latham WC (1966).** Active-passive tetanus immunization. *N Engl J Med. 274:186–90.*

113. **Levine L, Edsall G (1981).** Tetanus toxoid: what determines reaction proneness? *J Infect Dis. 144:376.*

114. **Lin TY, Wang YH, Chang LY, Chiu CH, Huang YC, Tang H 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 Med J. 26:315–22.*

115. **Lincetto O, Gomez P, Munjanja S (2006).** In: Opportunities for Africa's newborns: practical data, policy and programmatic support for newborn care in Africa. Lawn J, Kerber K, editors. Geneva: WHO on behalf of the Partnership for Maternal Newborn and Child Health. Section III, chapter 2. Antenatal care:51–62 (*http://www.who.int/pmnch/media/publications/aonsectionIII_2.pdf*, accessed 11 August 2018).

116. **Livorsi DJ, Eaton M, Glass J (2010).** Generalized tetanus despite prior vaccination and a protective level of anti-tetanus antibodies. *Am J Med Sci. 339(2):200–1.*

117. **Lloyd JC, Haber P, Mootrey GT, Braun MM, Rhodes PH, Chen RT; VAERS Working Group (2003).** Adverse event reporting rates following tetanus-diphtheria and tetanus toxoid vaccinations: data from the Vaccine Adverse Event Reporting System (VAERS) 1991–1997. *Vaccine. 21:3746–50.*

118. **Lodha R, Sareen A, Kumar RM, Arora NK (2000).** Tetanus in immunized children. *Indian Pediatr. 37:223–4.*

119. **Looney JM, Edsall G, Ipsen J, Chasen WH (1956).** Persistence of antitoxin levels after tetanus toxoid inoculation in adults, and effect of a booster dose after various intervals. *N Engl J Med. 254:6–12.*

120. **Luisto M, Iivanainen M (1993).** Tetanus of immunized children. *Dev Med Child Neurol. 35:351–5.*

121. **Macko MB (1985).** Comparison of the morbidity of tetanus toxoid boosters with tetanus-diphtheria toxoid boosters. *Ann Emerg Med. 14:33–5.*

122. **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:113–21.*

123. **MacLennan R, Schofield FD, Pittman M, Hardegree MC, Barile MF (1965).** Immunization against neonatal tetanus in New Guinea: antitoxin response of pregnant women to adjuvant and plain toxoids. *Bull World Health Organ. 32:683–97.*

124. **Maertens K, Caboré RN, Huygen K, Hens N, Van Damme P, Leuridan E (2016).** Pertussis vaccination during pregnancy in Belgium: results of a prospective controlled cohort study. *Vaccine. 34:142–50.*

125. **Mallet E, Belohradsky BH, Lagos R, Gothefors L, Camier P, Carrière JP et al.; Hexavalent Vaccine Trial Study Group (2004).** A liquid hexavalent combined vaccine against diphtheria, tetanus, pertussis, poliomyelitis. *Haemophilus influenzae* type B and hepatitis B: review of immunogenicity and safety. *Vaccine. 22:1343–57.*

126. **Maple PA, Jones CS, Wall EC, Vyseb A, Edmunds WJ, Andrews NJ et al. (2001).** Immunity to diphtheria and tetanus in England and Wales. *Vaccine. 19:167–73.*

127. **Martinón-Torres F, Boisnard F, Thomas S, Sadorge C, Borrow R; PRI02C Study Group (2017).** Immunogenicity and safety of a new hexavalent vaccine (DTaP5-IPV-HB-Hib) administered in a mixed primary series schedule with a pentavalent vaccine (DTaP5-IPV-Hib). *Vaccine. 35:3764–72.*

128. **Maselle SY, Matre R, Mbise R, Hofstad T (1991).** Neonatal tetanus despite protective serum antitoxin concentration. *FEMS Microbiol Immunol. 3:171–5.*

129. **Mathur GP, Singh YD, Mathur S, Chandra S (1980).** Tetanus neonatorum – its epidemiology and management. *Indian Pediatr. 17:797–801.*

130. **Matzkin H, Regev S (1985).** Naturally acquired immunity to tetanus toxin in an isolated community. *Infect Immun. 48:267–8.*

131. **Mayorga C, Torres MJ, Corzo JL, Alvarez J, García JA, Rodríguez CA et al. (2003).** Immediate allergy to tetanus toxoid vaccine: determination of immunoglobulin E and immunoglobulin G antibodies to allergenic proteins. *Ann Allergy Asthma Immunol. 90:238–43.*

132. **McCarroll J, Abrahams I, Skudder PA (1962).** Antibody response to tetanus toxoid 15 years after initial immunization. *Am J Public Health. 52:1669–75.*

133. **McCormick JB, Gusmão HH, Nakamura S, Freire JB, Veras J, Gorman G et al. (1980).** Antibody response to serogroup A and C meningococcal polysaccharide vaccines in infants born of mothers vaccinated during pregnancy. *J Clin Invest. 65:1141–4.*

134. **McQuillan GM, Kruszon-Moran D, Deforest A, Chu SY, Wharton M (2002).** Serologic immunity to diphtheria and tetanus in the United States. *Ann Intern Med. 136:660–6.*

135. **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. *J Biol Stand. 11:137–44.*

136. **Melvin AJ, Mohan KM (2003).** Response to immunization with measles, tetanus, and *Haemophilus influenzae* type b vaccines in children who have human immunodeficiency virus typ e 1 infection and are treated with highly active antiretroviral therapy. *Pediatrics. 111:641–4.*

137. **Menon PS, Sahai G, Joshi VB, Murthy RG, Boparai MS, Thomas AK (1976).** Field trial on frozen and thawed tetanus toxoid. *Indian J Med Res. 64:25–32.*

138. **Miller ER, Moro PL, Cano M, Shimabukuro TT (2015).** Deaths following vaccination: What does the evidence show? *Vaccine. 33:3288–92.*

139. **Monjour L, Bourdillon F, Schlumberger M, Fayet MT, Michon C. Ballet JJ et al. (1982).** Étude de l'immunité humor ale 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]. *Bull World Health Organ. 60:589–96.*

140. **Morgan JL, Baggari SR, McIntire DD, Sheffield JS (2015).** Pregnancy outcomes after antepartum tetanus, diphtheria, and acellular pertussis vaccination. *Obstet Gynecol. 125:1433–8.*

141. **Moss WJ, Clements CJ, Halsey NA (2003).** Immunization of children at risk of infection with human immunodeficiency virus. *Bull World Health Organ. 81:6170.*

142. **Mullaert J, Abgrall S, Lele N, Batteux F, Slama LB, Meritet JF et al.; ANRS VIHVO Study Group (2015).** Diphtheria, tetanus, poliomyelitis, yellow fever and hepatitis B seroprevalence among HIV1-infected migrants. Results from the ANRS VIHVO vaccine sub-study. *Vaccine. 33:4938–44.*

143. **Munoz FM, Bond NH, Maccato M, Pinell P, Hammill HA, Swamy GK et al. (2014).** Safety and immunogenicity of tetanus diphtheria and acellular pertussis (Tdap) immunization during pregnancy in mothers and infants: a ran domized clinical trial. *JAMA. 311:1760–9.*

144. **Myers MG, Beckman CW, Vosdingh RA, Hankins WA (1982).** Primary immunization with tetanus and diphtheria toxoids: reaction rate and immunogenicity in older children and adults. *JAMA. 248:2478–80.*

145. NHS Choices (website). Tetanus. London: National Health Service (*http://www.nhs.uk/conditions/Tetanus/Pages/Introduction.aspx*, accessed 11 August 2018).

146. **N'Diaye DS, Schwarzinger M, Obach D, Poissy J, Matheron S, Casalino E et al. (2014).** Effectiveness and cost of quick diagnostic tests to determine tetanus immunity in patients with a wound in French emergency departments. *BMC Infect Dis. 14:603.*

147. **Newell KW, Dueñas Lehmann A, LeBlanc DR, Garces Osorio N (1966).** The use of toxoid for the prevention of tetanus neonatorum. Final report of a double-blind controlled field trial. *Bull World Health Organ. 35:863–71.*

148. **Newell KW, Leblanc DR, Edsall G, Levine L, Christensen H, Montouri MH et al. (1971).** The serological assessment of a tetanus toxoid field trial. *Bull World Health Organ. 45:773–85.*

149. **Nohynek H, Gustafsson L, Capeding MR, Käyhty H, Olander RM, Pascualk L 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. *Pediatr Infect Dis J. 18:25–30.*

150. **Okoko BJ, Wesuperuma LH, Ota MO, Banya WA, Pinder M, Gomez FS 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. *J Health Popul Nutr. 19:59–65.*

151. **Okoko JB, Wesumperuma HL, Hart CA (2001).** The influence of prematurity and low birthweight on transplacental antibody transfer in a rural West African population. *Trop Med Int Health. 529–34.*

152. **Orimadegun AE, Orimadegun BE, Adepoju AA (2013).** Immunity against tetanus infection, risk factors for non-protection, and validation of a rapid immunotest kit among hospitalized children in Nigeria. *Front Neurol. 4:142.*

153. **Ouandaogo CR, Yaméogo TM, Diomandé FV, Sawadogo C, Ouédraogo B, Ouédraogo-Traoré R et al. (2012).** Adverse events following immunization during mass vaccination campaigns at first introduction of a meningococcal A conjugate vaccine in Burkina Faso, 2010. *Vaccine. 30(Suppl 2):B46–51.*

154. **Ourth DD, MacDonald AB (1977).** Neutralization of tetanus toxin by human and rabbit immunoglobulin classes and subunits. *Immunology. 33:807–15.*

155. **Owa JA, Makinde OO (1990).** Neonatal tetanus in babies of immunized mothers. *J Trop Pediatr. 36:143–4.*

156. **Oyedeyji GA, Olamijulo SK, Joiner KT (1982).** Neonatal tetanus in Ilesa, Nigeria: a review of the present status. *Niger Med J. 12:131–5.*

157. **Paulke-Korinek M, Rendi-Wagner P, Kundi M, Tomann B, Wiedermann U, Kollaritsch H (2008).** Pretravel consultation: rapid dipstick test as a decision guidance for the application of tetanus booster vaccinations. *J Travel Med. 15:437–41.*

158. **Pascual FB, McGinley EL, Zanardi LR, Cortese MM, Murphy TV (2003).** Tetanus surveillance – United States, 1998–2000. MMWR. *CDC surveillance summaries:52:1–8.*

159. **Passen EL, Andersen BR (1986).** Clinical tetanus despite a protective level of toxin-neutralizing antibody. *JAMA. 255:1171–3.*

160. **Peel MM (1980).** Measurement of tetanus antitoxin. II. Toxin neutralization. *J Biol Stand. 8:191–207.*

161. **Perry AL, Hayes AJ, Cox HA, Alcock F, Parker AR (2009).** Comparison of five commercial anti-tetanus toxoid immunoglobulin G enzyme-linked immunosorbent assays. *Clin Vaccine Immunol. 16:1837–9.*

162. **Pickering JW, Martins TB, Schroder MC, Hill HR (2002).** Comparison of a multiplex flow cytometric assay with enzym e-linked immuno sorbent assay for quantitation of antibodies to tetanus, diphtheria and *Haemophilus influenzae* type b. *Clin Diagn Lab Immunol. 9:872–6.*

163. **Pirouzmand H, Khameneh B, Tafaghodi M (2017).** Immunoadjuvant potential of cross-linked dextran microspheres mixed with chitosan nanospheres encapsulated with tetanus toxoid. *Pharm Biol. 55:212–7.*

164. **Pryor T, Onarecker C, Coniglione T (1997).** Elevated antitoxin titers in a man with generalized tetanus. *J Fam Pract. 44:299–303.*

165. **PHE (2013).** Immunisation against infectious disease: the green book. London: Public Health England (*https://www.gov.uk/government/organisations/public-health-england/series/immunisation-against-infectious-disease-the-green-book*, accessed 9 August 2018).

166. **Rahman M, Chen LC, Chakraborty J, Yunus M, Chowdhury AI, Sarder AM 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. *Bull World Health Organ. 60:261–7.*

167. **Ramakrishnan G, Wright M, Alam M, Naylor C, Kabir M, Zerin A et al. (2017).** Rapid assessment of tetanus vaccine-induced immunity in Bangladesh and the Gambia. *Diagn Microbiol Infect Dis. 87:272–4.*

168. **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. 44:1625–8.*

169. **Ramsay ME, Rao M, Begg NT, Redhead K, Attwell AM (1993).** Antibody response to accelerated immunization with diphtheria, tetanus, pertussis vaccine. *Lancet. 342:203–5.*

170. **Ratliff D A, Burns-Cox CJ (1983).** Anaphylaxis to tetanus toxoid (unreviewed reports). *BMJ. (Clinical Research Edition). 288:114.*

171. **Redwan ERM, Al-Awady MK (2002).** Prevalence of tetanus immunity in the Egyptian population. *Human Antibodies. 11:55–9.*

172. **Relihan M (1969).** Reactions to tetanus toxoid. *Ir Med J. 62:430–4.*

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

174. **Richmond P, Borrow R, Findlow J, Martin S, Thornton C, Cartwright K 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. *Infect Immun. 69:2378–82.*

175. **Roper MH, Wassilak SGF, Scobie HM, Ridapath AD, Orenstein W (2017).** Tetanus toxoid. In: Plotkin SA, Orenstein WA, Offit PA, Edwards KM, editors. *Vaccines, seventh edition:1052–79.*

176. **Roper MH , Wassilak SGF, Scobie HM, Ridapath AD, Orenstein W (2013).** Tetanus toxoid. In: Plotkin SA, Orenstein WA, Offit PA, editors. *Vaccines, sixth edition:746–72.*

177. **Rosen JB, Breman JG (2004).** Malaria intermittent preventative treatment in infants, chemoprophylaxis, and childhood vaccinations. *Lancet. 363:1386–8.*

178. **Rosenblatt HM, Song LY, Nachman SA, Stanley KE, Krogstad PA, Johnson GM et al.;** Pediatric Aids Clinical Trials Group 377 Study Team (2005). Tetanus immunity after diphtheria, tetanus toxoids and acellular pertussis vaccination in children with clinically stable HIV infection. *J Allergy Clin Immunol. 116:698–703.*

179. **Ryder RW, Oxtoby MJ, Mvula M, Batter V, Baende E, Nsa W 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. *J Pediatr. 122:697–702.*

180. **Sachs A (1952).** Modern views on prevention of tetanus in wound ed. *Proceedings of the Royal Society of Medicine. 45:641–52.*

181. **Saenger R, Maechler G, Potreck M, Zepp F, Knuf M, Habermehl P 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. 23:1135–43.*

182. **Sangpetchsong V, Impat A, Dhiensiri K, Podhipak A (1985).** Effect of passive immunity to tetanus in DTP vaccinated infants. *Southeast Asian J Trop Med Public Health. 16:117–23.*

183. **Sarvas H, Seppälä I, Kurikka S, Siegberg R, Mäkelä O (1993).** Half-life of the maternal IgG1 allotype in infants. *J Clin Immunol. 13:145–51.*

184. **Sauerbrei A, Prager J, Bischoff A, Wutzler P (2004).** [Antibodies against vaccine-preventable diseases in pregnant women and their offspring. Diphtheria, pertussis, tetanus]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 47:16–20.*

185. **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.*

186. **Schlumberger M, Yvonnet B, Lesage G, Tep B (2015).** Low specificity of 2 tetanus rapid tests in Cambodia. *Médecine et Maladies Infectieuses. 45:29–33.*

187. **Schofield FD, Tucker VM, Westbrook GR (1961).** Neonatal tetanus in New Guinea. *BMJ. 2:785–9.*

188. **Scobie HM, Mao B, Buth S, Wannemuehler KA, Sørensen C, Kannarath C et al. (2016).** Tetanus immunity among women aged 15 to 39 years in Cambodia: a national population-based serosurvey, 2012. *Clin Vaccine Immunol. 23:546–54.*

189. **Scobie HM, Patel M, Martin D, Mkocha H, Njenga SM, Odiere MR et al. (2017).** Tetanus immunity gaps in children 5–14 years and men ≥15 years of age revealed by integrated disease serosurveillance in Kenya, Tanzania, and Mozambique. *Am J Trop Med Hyg. 96:415–20.*

190. **Scott S, Cumberland P, Shulman CE, Cousens S, Cohen BJ, Brown DW 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. *J Infect Dis. 191:1854–60.*

191. **Sedgwick AK, Ballow M, Sparks K, Tilton RC (1983).** Rapid quantitative microenzyme-linked immunosorbent assay for tetanus antibodies. *J Clin Microbiol. 18:104–9.*

192. **Sesardic D, Wong MY, Gaines Das RE, Corbel MJ (1993).** The first international standard for anti-tetanus immunoglobulin, human: pharmaceutical evaluation and international collaborative study. *Biologicals. 21:67–75.*

193. **Shumacker HB Jr, Lamont A (1942).** Some problems concerning active immunization against tetanus. *Surg Gynecol Obstet. 74:433–8.*

194. **Siber GR (1997).** Methods for estimating serological correlates of protection. *Dev Biol (Basel). 89:283–96.*

195. **Simonsen O, Bentzon MW, Kjeldsen K, Venborg HA, Heron I (1987a).** Evaluation of vaccination requirements to secure continuous antitoxin immunity to tetanus. *Vaccine. 5:115–21.*

196. **Simonsen O, Klaerke M, Jensen JE, Kjeldsen K, Hau C, Heron I (1987c).** Revaccination against tetanus 17 to 20 years after primary vaccination: Kinetics of antibody response. *J Trauma. 27:1358–61.*

197. **Simonsen O, Bentzon MW, Heron I (1986).** ELISA for the routine determination of antitoxic immunity to tetanus. *J Biol Stand. 14:231–239.*

198. **Simonsen O, Schou C, Heron I (1987b).** Modification of the ELISA for the estimation of tetanus antitoxin in human sera. *J Biol Stand. 15:143–57.*

199. **Sisk C W, Lewis C E (1965).** Reactions to tetanus-diphtheria toxoid (adult). *Arch Environ Health. 11:34–6.*

200. **Sneath PAT, Kerslake EG (1935).** Persistence of tetanus antitoxin in man following active immunization. *BMJ. 2:290.*

201. **Sneath PAT, Kerslake E G, Sruby F (1937).** Tetanus immunity: resistance of guinea pigs to lethal spore doses induced by active and passive immunization. *Am J Hyg. 25:464–76.*

202. **Sonobe MH, Trezena AG, Guilhen FB, Takano VL, Fratelli F, Sakauchi D, et al. (2007).** Determination of low tetanus or diphtheria antitoxin titers in sera by a toxin neutralization assay and a modified toxin-binding inhibition test. *Braz J Med Biol Res. 40:69-76.*

203. **Sodha SV, Dietz V (2015).** Strengthening routine immunization systems to improve global vaccination coverage. *Br Med Bull. 113:5–14.*

204. **Stanfield JP, Gall D, Bracken PN (1973).** Single dose antenatal tetanus immunization. *Lancet. 1:215–9.*

205. **Stark K, Schönfeld C, Barg J, Molz B, Vornwald A, Bienzle U (1999).** Seroprevalence and determinants of diphtheria, tetanus and poliomyelitis among adults in Berlin, Germany. *Vaccine. 17:844–50.*

206. **Stiffler-Rosenberg G, Fey H (1975).** Radioimmunologische Messung von Tetanusantitoxin [Radioimmunological measurement of tetanus antitoxin]. *Schweizerische Medizinische Wochenschrift. 105:804–10.*

207. **Stiffler-Rosenberg G, Fey H. (1977).** [Measurement of human tetanus antitoxin using as enzyme-linked immunosorbent assay]. *Schweizerische medizinische Wochenschrift. 107:1101-4.*

208. **Steens A, Mollema L, Berbers GA, van Gageldonk PG, van der Klis FR, de Melker HE (2010).** High tetanus antitoxin antibody concentrations in the Netherlands: a seroepidemiological study. *Vaccine. 28:7803–9.*

209. **Stroffolini T, Giammanco A, Giammanco G, Taormina S, Maggio M, Genovese M et al. (1997).** Immunity to tetanus in the 3–20-year age group in Italy. *Public Health. 111:19–21.*

210. **Stubbe M, Swinnen R, Crusiaux A, Mascart F, Lheureux PE (2007).** Seroprotection against tetanus in patients attending an emergency department in Belgium and evaluation of a bedside immunotest. *Eur J Emerg Med. 14:14–24.*

211. **Sukumaran L, McCarthy NL, Kharbanda EO, McNeil MM, Naleway AL, Klein NP et al. (2015).** Association of Tdap vaccination with acute events and adverse birth outcomes among pregnant women with prior tetanus-containing immunizations. *JAMA. 314:1581–7.*

212. **Suri JC, Rubbo SD (1961).** Immunization against tetanus. *J Hyg (Lond). 59(1):29–48.*

213. **Swartz TA, Saliou P, Catznelson E, Blondeau C, Gil I, Peled T 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. *Eur J Epidemiol. 18:827–33.*

214. **Symeonidis N, Symeonidis C, Souliou E, Houiazi E, Diza E, Symeonidis A et al. (2003).** Serological survey of immunity to tetanus in adult population of Nothern Halkidiki, Greece. *Eur J Epidemiol., 18:1147–52.*

215. **Talbot EA, Brown KH, Kirkland KB, Baughman AL, Halperin SA, Broder KR. (2010).** The safety of immunizing with tetanus-diphtheria-acellular pertussis vaccine (Tdap) less than 2 years following previous tetanus vaccination: experience during a mass vaccination campaign of health care personnel during a respiratory illness outbreak. *Vaccine. 28:8001–7.*

216. **Tapia MD, Sow SO, Haidara FC, Diallo F, Doumbia M, Enwere GC et al. (2015).** A Phase 3, Double-Blind, Randomized, Active Controlled Study to Evaluate the Safety of MenAfriVac in Healthy Malians. *Clin Infect Dis. 61 (Suppl 5):S507–13.*

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

218. **Tenbroeck C, Bauer JH (1923).** Studies on the relation of tetanus bacilli in the digestive tract to tetanus antitoxin in the blood. *J Exp Med. 37:479–89.*

219. **National Academies Press (2014).** Adverse effects of vaccines: evidence and causality. Institute of Medicine report. Washington (DC): National Academies Press (*https://www.nap.edu/download/13164*, accessed 11 August 2018).

220. **Theeten H, Hutse V, Hens N, Yavuz Y, Hoppenbrouwers K, Beutels P et al. (2011).** Are we hitting immunity targets? The 2006 age-specific seroprevalence of measles, mumps, rubella, diphtheria and tetanus in Belgium. *Epidemiol Infect. 139:494–504.*

221. **Tomovici A, Barreto L, Zickler P, Meekison W, Noya F, Voloshen T et al. (2012).** Humoral immunity 10 years after booster immunization with an adolescent and adult formulation combined tetanus, diphtheria, and 5-component acellular pertussis vaccine. *Vaccine. 30:2647–53.*

222. **Turner TB, Stafford ES, Goldman L (1954).** Studies on the duration of protection afforded by active immunization against tetanus. *Bulletin of the Johns Hopkins Hospital. 86:205–17.*

223. **Vandelaer J, Birmingham M, Gasse F, Kurian M, Shaw C, Garnier S (2003).** Tetanus in developing countries: an update on the maternal and neonatal tetanus elimination initiative. *Vaccine. 21:3442–5.*

224. **van der Maas NA, Donken R, Te Wierik MJ, Swaan CM, Hahne SJ, de Melker HE (2016).** Performance of a bedside test for tetanus immunity: results of a cross-sectional study among three EDs in the Netherlands in 2012–2013. *Emerg Med J. 33:763–8*

225. **van Gageldonk PG, van Schaijk FG, van der Klis FR, Berbers GA (2008).** Development and validation of a multiplex immunoassay for the simultaneous determination of serum antibodies to Bordetella pertussis, diphtheria and tetanus. *J Immunol Methods. 335:79–89.*

226. **van Hoeven KH, Dale C, Foster P, Body B (2008).** Comparison of three enzyme-linked immunosorbent assays for detection of immunoglobulin g antibodies to tetanus toxoid with reference standards and the impact on clinical practice. *Clin Vaccine Immunol. 15:1751–4.*

227. **Vannice KS, Keita M, Sow SO, Durbin AP, Omer SB, Moulton LH et al. (2015).** Active surveillance for adverse events after a mass vaccination campaign with a group A meningococcal conjugate vaccine (PsA-TT) in Mali. *Clin Infect Dis. 61(Suppl 5):S493–500.*

228. **Vergara R, Tregnaghi M, Ussher J, Navarro S, Rüttimann R, Potin M 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. *Eur J Pediatr. 164:377–82.*

229. **Verma M, Dhanwade SM, Singh T (1989).** Clinical study of tetanus neonatorum. *Indian J Med Sci 43:235–8.*

230. **Veronesi R, Correia A, Ferreira H (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:613–26.*

231. **Veronesi R, Bizzini B, Focaccia R, Coscina AL, Mazza CC, et al. (1983).** Naturally acquired antibodies to tetanus toxin in humans and animals from the galápagos islands. J Infect Dis. 147:308-11. Virella G, Hyman B (1991). Quantitation of anti-tetanus and anti-diphtheria antibodies by enzymoimmunassay: methodology and applications. *J Clin Lab Anal. 5:43–8.*

232. **Volk V, Gottshall RY, Anderson HD, Top FH, Bunney WE, Serfling RE (1962).** Antigenic response to booster dose of diphtheria and tetanus toxoids. *Public Health Rep. 77:185–94.*

233. **von Reyn CF, Clements CJ, Mann JM (1987).** Human immunodeficiency virus infection and routine childhood immunisation. *Lancet. 2:669–72.*

234. **Voysey M, Kandasamy R, Yu LM, Baudin M, Sadorge C, Thomas S et al. (2016).** The predicted persistence and kinetics of antibody decline 9years after pre-school booster vaccination in UK children. *Vaccine. 34:4221–8.*

235. **Voysey M, Kelly DF, Fanshawe TR, Sadarangani M, O'Brien KL, Perera R et al. (2017).** The influence of maternally derived antibody and infant age at vaccination on infant vaccine responses: an individual participant meta-analysis. *JAMA Pediatrics. 171:637–46.*

236. **Wagner KS, White JM, Andrews NJ, Borrow R, Stanford E, Newton E et al. (2012).** Immunity to tetanus and diphtheria in the UK in 2009. *Vaccine. 30:7111–7.*

237. **Wang AS, Burns GF, Kronborg IJ, Mackay IR. (1982).** Detection of antibodies to tetanus toxoid: comparison of a direct haemagglutination method with a radioimmunoassay. *J Clin Path. 35:1138-41.*

238. **Wassilak SGF, Roper MH, Scobie HM, Ridapath AD, Orenstein W (2004).** Tetanus toxoid. In: Plotkin SA, Orenstein WA, editors. V*accines, fourth edition. Philadelphia (PA): WB Saunders:745–81.*

239. **White W, Barnes GM, Barker E, Gall D, Knight P, Griffith AH et al. (1973).** Reactions to tetanus toxoid. *J Hyg (Lond). 71(2):283–97.*

240. **Wolters KL, Dehmel H (1938).** Ûber den verlauf der aktiven und aktiv-passiven immunität bei tetanus [On the course of active and active-passive immunity against tetanus]. *Zeitschrift J. Infektionskr. 53:140–7.*

241. **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. 124:326–332.*

242. World Health Assembly (1989). Resolution WHA42.32 on Expanded Programme on Immunization. Forty-second World Health Assembly, Geneva, 8–19 May 1989: resolutions and decisions, annexes (*http://apps.who.int/iris/handle/10665/171211*, accessed 11 August 2018).

243. WHO (1982). Expanded Programme on Immunization. Prevention of neonatal tetanus. Wkly Epidemiol Rec. 57:137–42 (*http://apps.who.int/iris/bitstream/10665/223963/1/WER5718_137-142.PDF*, accessed 11 August 2018).

244. WHO (1996a). Expanded Programme on Immunization. Estimating tetanus protection of women by serosurvey. Wkly Epidemiol Rec. 71:117–24 (*http://apps.who.int/iris/bitstream/10665/229744/1/WER7116_117-120.PDF*, accessed 11 August 2018).

245. WHO (1996b). Expanded Programme on Immunization. The Children's Vaccine Initiative and the Global Programme for Vaccines and Immunization. Part I. Wkly Epidemiol Rec. 71:261–8 (http://apps.who.int/iris/bitstream/10665/229879/1/WER7135_261-266.PDF, accessed 11 August 2018).

246. WHO (1996c). Expanded Programme on Immunization. Progress towards neonatal tetanus elimination, 1988–1994. Wkly Epidemiol Rec. 71:33–6 (http://apps.who.int/iris/bitstream/10665/229665/1/WER7105_33-36.PDF, accessed 11 August 2018).

247. WHO (1999a). Expanded Programme on Immunization. Progress towards the global elimination of neonatal tetanus, 1990–1998. Wkly Epidemiol Rec. 74:73–80 (http://apps.who.int/iris/bitstream/10665/230729/1/WER7410_73-80.PDF, accessed 11 August 2018).

248. WHO (2001). Expanded Programme on Immunization. Assessment of elimination of neonatal tetanus, Zimbabwe. Wkly Epidemiol Rec. 2001, 76:101–6 (http://apps.who.int/iris/bitstream/10665/231474/1/WER7614_101-106.PDF, accessed 11 August 2018).

249. WHO (2002). Expanded Programme on Immunization. Assessment of neonatal tetanus elimination, Namibia. Wkly Epidemiol Rec. 77:121–3 (http://apps.who.int/iris/bitstream/10665/231829/1/WER7715_121-123.PDF , accessed 11 August 2018).

250. WHO (1999b). Field manual for neonatal tetanus elimination. (Document WHO/V&B/99.14). Geneva: World Health Organization (http://apps.who.int/iris/bitstream/10665/83323/1/WHO_V-B_99.14_eng.pdf, accessed 11 August 2018).

251. WHO, UNICEF, UNFPA (2002). Maternal and neonatal tetanus elimination by 2005: strategies for achieving and maintaining elimination, November 2000. (Document WHO/V&B/02.09). Geneva: World Health Organization, United Nations Children's Fund, United Nations Population Fund (https://www.unicef.org/health/files/MNTE_strategy_paper.pdf, accessed 11 August 2018).

252. WHO (2006). Temperature sensitivity of vaccines. (Document WHO/IVB/06.10). Geneva: World Health Organization (http://apps.who.int/iris/bitstream/10665/69387/1/WHO_IVB_06.10_eng.pdf, accessed 11 August 2018).

253. WHO (2011). Validation of neonatal tetanus elimination in Burundi and the Comoros by a lot quality assurance cluster sampling survey, 1 October 2008–30 September 2009. Wkly Epidemiol Rec. 8;86(28):289–99.

254. WHO (2016a). Meeting of the Strategic Advisory Group of Experts (SAGE) on Immunization. Geneva: World Health Organization (http://www.who.int/immunization/sage/meetings/2016/october/presentations_background_docs/en/, accessed 11 August 2018).

255. WHO (2016b). Strategic Advisory Group of Experts (SAGE) on Immunization. Report of the SAGE Working Group on Maternal and Neonatal Tetanus Elimination and Broader Tetanus Prevention. Geneva: World Health Organization (*http://www.who.int/immunization/sage/meetings/2016/october/1_Report_of_the_SAGE_Working_Group_on_Maternal_and_Neonatal_Tetanus_27Sep2016.pdf*, accessed 11 August 2018).

256. WHO (2016c). Report of the WHO Technical Advisory Group on Innovations in Male Circumcision – consultative review of additional information. Geneva: World Health Organization (*http://apps.who.int/iris/bitstream/handle/10665/250146/WHO-HIV-2016.19-eng.pdf?sequence=1*, accessed 11 August 2018).

257. WHO (2017b). Maternal and neonatal tetanus elimination (MNTE) (website). Geneva: World Health Organization (*http://www.who.int/immunization/diseases/MNTE_initiative/en/*, accessed 11 August 2018).

258. WHO (2017c). Tetanus toxoid vaccination and voluntary medical male circumcision (website). Geneva: World Health Organization (*http://www.who.int/immunization/programmes_systems/interventions/TT_and_VMMC/en/*, accessed 11 August 2018).

259. WHO (2017a). Tetanus vaccines: WHO position paper. Wkly Epidemiol Rec. 6(92):53–76 (*http://apps.who.int/iris/bitstream/10665/254582/1/WER9206.pdf?ua=1*, accessed 11 August 2018).

260. WHO (2018). Tetanus vaccines: WHO position paper, February 2017 – Recommendations. *Vaccine. 36:3573–5.*

261. WHO Vaccine Safety Basics. E-learning course (website). Module 1: Introduction to vaccine safety. Geneva: World Health Organization (*http://vaccine-safety-training.org/adverse-events-causes.html*, accessed 11 August 2018).

262. **Yuan L, Lau W, Thipphawong J, Kasenda M, Xie F, Bevilacqua J (1997).** Diphtheria and tetanus immunity among blood donors in Toronto. *Can Med Assoc J. 156:985–90.*

263. **Zalogna GP, Chernow B (1982).** Life-threatening anaphylactic reactions to tetanus toxoid. *Ann Allergy. 48:107–8.*

264. **Zuger B, Greenwald CK, Gerber H (1942).** Tetanus immunization: effectiveness of stimulating dose of toxoid under conditions of infection. *J Immunol. 44:309–17.*

9 789241 513616

Department of Immunization, Vaccines and Biologicals

World Health Organization
20, Avenue Appia
CH-1211 Geneva 27, Switzerland
vaccines@who.int
http://www.who.int/immunization/en/

World Health Organization