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Recommendations to assure the quality, safety and efficacy of typhoid conjugate vaccines

Replacement of Annex 3 of WHO Technical Report Series, No. 987

Adopted by the Seventy-second meeting of the World Health Organization Expert Committee on Biological Standardization, 19–23 October 2020. A definitive version of this document, which will differ from this version in editorial but not scientific details, will be published in the WHO Technical Report Series.

Annex 2

Recommendations to assure the quality, safety and efficacy of typhoid conjugate vaccines

Replacement of Annex 3 of WHO Technical Report Series, No. 987

Recommendations published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes recommendations for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these WHO Recommendations are made only on condition that such modifications ensure that the product is at least as safe and efficacious as that prepared in accordance with the recommendations set out below.

Abbreviations

ADH	Adipic acid dihydrazide
C. freundii s.l.	*Citrobacter freundii* sensu lato
CI	confidence interval
CRM_{197}	cross-reactive material 197
CTAB	hexadecyltrimethylammonium bromide
DT	diphtheria toxoid
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (also abbreviated to EDAC)
ELISA	enzyme-linked immunosorbent assay
HPAEC-CD	high-performance anion exchange chromatography with conductivity detection
HPAEC-PAD	high-performance anion exchange chromatography with pulsed amperometric detection
HPLC	high-performance liquid chromatography
HPSEC	high-performance size-exclusion chromatography
IgA	immunoglobulin A
IgG	immunoglobulin G
IU	International Unit
K_D	(distribution constant)
LAL	*Limulus* amoebocyte lysate (test)
LPS	lipopolysaccharide
MAT	monocyte activation test
MW	molecular weight
NCL	national control laboratory
NMR	nuclear magnetic resonance
NRA	national regulatory authority
qNMR	quantitative nuclear magnetic resonance
SAGE	WHO Strategic Advisory Group of Experts
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
S. Typhi	*Salmonella enterica* serovar Typhi
TCV	typhoid conjugate vaccine
TT	tetanus toxoid
Vi-rEPA	Vi polysaccharide conjugated to recombinant exoprotein A of *Pseudomonas aeruginosa*

Introduction

The WHO Guidelines on the quality, safety and efficacy of typhoid conjugate vaccines were developed following a series of international consultations in 2012 and 2013, and were adopted by the WHO Expert Committee on Biological Standardization at its Sixty-fourth meeting in October 2013 *(1)*. Since that time, there have been several major developments with respect to typhoid conjugate vaccines (TCVs), including:

- The establishment of WHO international standards for Vi antigens and Vi antibodies (human).
- The licensing of TCVs in some countries.
- The publication of a WHO Strategic Advisory Group of Experts (SAGE) position paper in 2018 recommending the use of TCVs from 6 months to 45 years of age, and that the introduction of TCVs into routine immunization programmes be prioritized in countries with the highest burden of typhoid disease or with a high burden of antimicrobial-resistant *Salmonella* Typhi.
- Approval of funding support by Gavi, the Vaccine Alliance, for the introduction of TCVs in Gavi-eligible countries starting in 2019.
- WHO Prequalification in 2017 of the Typbar-TCV[1] produced by a manufacturer in India.

The impact of these developments on the production and quality control of TCVs and on their nonclinical and clinical evaluation is reflected in the present revision. As TCVs have been licensed since the development of the original WHO Guidelines in 2013, the current document provides recommendations for the evaluation of such vaccines rather than guiding principles. As a consequence of the increasing demand for TCVs, together with the above-mentioned Gavi decision on funding, new vaccine developers and manufacturers are entering the field and should benefit from updated WHO recommendations. Further clinical evaluation of TCVs, the detailed investigation of immune responses to these vaccines and the search for a true immunological correlate or surrogate of protection are ongoing and Part C of this document may therefore require further updating as new data become available.

Other significant changes reflected in the current document include the updating in 2017 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations *(2)* which provide methodological considerations for the clinical evaluation of vaccine immunogenicity, efficacy and safety. Manufacturers and regulators should also take note of the decision of the Committee in 2018 to discontinue the inclusion of the general safety (innocuity) test in routine lot release testing requirements for all vaccines in WHO Recommendations, Guidelines and other guidance documents for biological products *(3)*. This test is therefore not included in the manufacturing recommendations provided in Part A of the current document.

Terminology

The definitions given below apply to the terms as used in these WHO Recommendations. These terms may have different meanings in other contexts.

Activated carrier protein: a carrier protein that has been chemically or physically modified and prepared for conjugation to the polysaccharide.

[1] See: WHO Prequalified Vaccines at: https://extranet.who.int/gavi/PQ_Web/

Activated polysaccharide: purified polysaccharide that has been modified by a chemical reaction or a physical process in preparation for conjugation to the activated carrier protein.

Carrier protein: the protein to which the Vi polysaccharide is covalently linked for the purpose of eliciting a T-cell-dependent immune response to the Vi polysaccharide.

Final bulk: the homogeneous preparation from one or more lots of **purified bulk conjugate** in a single container from which the final containers are filled, either directly or through one or more intermediate containers.

Final lot: a number of sealed, final containers that are equivalent with respect to the risk of contamination that may have occurred during filling and, when it is performed, freeze-drying. A final lot must therefore have been filled from a single container and if freeze-dried this should be completed in one continuous working session.

Master seed lot: bacterial suspensions for the production of Vi polysaccharide or the carrier protein should be derived from a strain that has been processed as a single lot and is of uniform composition. The master seed lot is used to prepare the **working seed lots**. Master seed lots should be maintained in the freeze-dried form or be frozen at or below −45 °C.

Purified bulk conjugate: a purified bulk conjugate is prepared by the covalent bonding of activated Vi polysaccharide to the carrier protein, followed by the removal of residual reagents and reaction by-products. This is the parent material from which the **final bulk** is prepared.

Purified polysaccharide: the material obtained after final purification of polysaccharide. The lot of purified polysaccharide may be derived from a **single harvest** or a pool of single harvests that have been processed together.

Single harvest: the material obtained from one batch of culture that has been inoculated with the **working seed lot** (or with the inoculum derived from it), harvested and processed during one production run.

Working seed lot: a quantity of bacterial suspension for the production of Vi polysaccharide or the carrier protein that is of uniform composition and that has been derived from the **master seed lot** by growing the organisms and maintaining them in freeze-dried aliquots or frozen at or below −45 °C. The working seed lot is used to inoculate the production medium.

General considerations

Typhoid fever is an acute generalized infection of the mononuclear phagocyte system (previously known as the reticuloendothelial system), intestinal lymphoid tissue and gall bladder caused by *Salmonella enterica* serovar Typhi (*S.* Typhi). Paratyphoid fever is a clinically indistinguishable illness caused by *S. enterica* serovar Paratyphi A or B (or, more rarely, C) *(4–6)*. Typhoid and paratyphoid fevers are referred to collectively as enteric fever. In most endemic areas, typhoid accounts for approximately 75–80% of cases of enteric fever. However, in some regions, particularly in some parts of Asia, *S.* Paratyphi A accounts for a relatively larger proportion of all enteric fevers *(7–9)*. Prospective vaccines against *S.* Paratyphi are not included in the scope of the current document.

Pathogen

S. Typhi is a member of the family *Enterobacteriaceae*. It is a Gram-negative, non-lactose fermenting bacillus that produces trace amounts of hydrogen sulfide. Its antigens include an immunodominant lipopolysaccharide (LPS) O9, flagellar H phase 1 antigen “d” and capsular polysaccharide Vi.

Vi acts as a virulence factor by preventing anti-O antibody from binding to the O antigen *(10)* and inhibits the C3 component of the complement system from fixing to the surface of *S.* Typhi *(11).* The Vi antigen is not unique to *S.* Typhi – it is also expressed by *S.* Paratyphi C, *Citrobacter freundii* sensu lato (*C. freundii* s.l.) and some clades of *S. enterica* serovar Dublin. The genes responsible for the biosynthesis of Vi polysaccharide are located in a locus (*viaB*) within the Salmonella pathogenicity island 7 (SPI-7) in the *S.* Typhi chromosome. Several other loci participate in the complex regulation of Vi expression. Almost all *S.* Typhi isolates from blood cultures express Vi. Nevertheless, Vi-negative strains have been identified occasionally, both in sporadic cases as well as during outbreaks *(12).* Some of these strains are regulatory mutants that can revert to a Vi-positive state *(13).* However, some Vi-negative isolates from blood have been shown to harbour deletion mutations in critical genes (for example, *tviB*) within the *viaB* locus that render the strains unable to synthesize Vi. This raises the theoretical concern that large-scale usage of Vi-containing vaccines (either polysaccharide or conjugate) could lead to selective pressure that creates a biological advantage for the emergence of Vi-negative strains *(14).*

Pathogenesis

Typhoid infection begins with ingestion of *S.* Typhi in contaminated food or water. In the small intestine, the bacteria penetrate the mucosal layer and ultimately reach the lamina propria. Translocation from the intestinal lumen mainly occurs through *S.* Typhi targeting M cells overlying gut-associated lymphoid tissue. Within this lymphoid tissue and in the lamina propria, *S.* Typhi invokes an influx of macrophages and dendritic cells that ingest the bacteria but fail to destroy them. Thus, some bacteria remain within macrophages in the lymphoid tissue of the small intestine and flow into the mesenteric lymph nodes where there is an inflammatory response mediated by the release of various cytokines. Bacteria enter the bloodstream via lymphatic drainage, thereby seeding organs of the mononuclear phagocyte system (such as the spleen, liver and bone marrow) and gall bladder by means of a silent primary bacteraemia. After a typical incubation period of 8–14 days the clinical illness begins, usually with the onset of fever, abdominal discomfort and headache. An accompanying low-level secondary bacteraemia occurs.

Prior to the availability of fluoroquinolone antibiotics, clinical relapses were observed in 5–30% of patients treated with antibacterial agents such as chloramphenicol and sulfamethoxazole/trimethoprim. These post-treatment relapses occurred when typhoid bacilli re-emerged from their protected intracellular niches within the macrophages of the mononuclear phagocyte system, where the antibacterial agents could not penetrate.

Several lines of investigation indicate that in a small proportion of patients infected with *S.* Typhi who may have premorbid abnormalities of the gall bladder mucosa (such as occurs consequent to gallstones) gall bladder infection becomes chronic (that is, excretion lasts for longer than 12 months) *(15).* Such chronic carriers, who are themselves not clinically affected by the presence of typhoid bacilli in their system, may excrete the pathogen in their faeces for decades *(16).* They are thought to serve as a long-term epidemiological reservoir in the community, and to foster the transmission of typhoid wherever there is inadequate sanitation, untreated water supplies and/or improper food handling.

Epidemiology

Typhoid fever is restricted to human hosts and in the late nineteenth and early twentieth century was endemic in virtually all countries in Europe and the Americas. Subsequently, the widespread use of chlorination, sand filtration and other means of water treatment drastically

reduced the incidence of typhoid fever despite the high prevalence of chronic carriers *(15)*. Typhoid remains endemic in most developing countries and is an important public health problem mainly because large segments of the population lack access to safe water and basic sanitation services *(17)*. In addition, there are limited programmes for detecting carriers and restricting them from handling food.

Disease burden

Varied estimates of the annual epidemiological burden (incidence and total number of cases) of typhoid fever have been published in the scientific literature based on the extrapolation of data from various sources. The true incidence of typhoid fever in most regions of developing countries is not known. One study published in 2004 estimated that ~22 million cases occur each year causing 216 000 deaths, predominantly in school-age children and young adults; annual incidence was estimated to be 10–100 per 100 000 population *(18)*. A systematic review of population-based studies published between 1984 and 2005 indicated an annual incidence of 13–976 per 100 000 population each year based on diagnosis by blood culture *(19)*.

More recent analysis has shown that typhoid fever remains a major cause of enteric disease of children in low- and middle-income countries, with global estimates of disease burden ranging from 11 to 21 million typhoid fever cases and approximately 145 000 to 161 000 deaths annually *(20)*. The majority of cases occur in Asia and sub-Saharan Africa but many of the island nations of Oceania also experience a moderate to high incidence of typhoid fever and large outbreaks *(21)*.

Several factors affect the calculation of the burden of typhoid disease, including the way in which the information is obtained. For example, data on the incidence of typhoid, its age-specific distribution and the severity of clinical disease obtained from passive surveillance implemented at health facilities can differ from data acquired through active surveillance. During active surveillance, households are visited systematically once or twice a week to detect fever among household members, and mild or early clinical illness can be detected. One of the most important factors however is how to confirm that a patient with acute febrile illness has typhoid fever. Unfortunately, there is no rapid, affordable and accurate point-of-care or laboratory diagnostic test to confirm a case of acute typhoid fever. Bone marrow culture is widely recognized as the gold standard but is impractical for widespread use. Blood culture is the most practical accurate confirmatory test but its use alone identifies only 40–80% of the cases that are detectable using bone marrow culture *(22–24)*. Cultures of bile containing duodenal fluid and of skin snips of rose spots can be positive when blood cultures are negative *(19)*. Prior patient treatment with antibacterial agents and the volume of blood cultured also affect the yield of cultures. Reliance on clinical diagnosis alone is not advisable because several other febrile syndromes caused by other microorganisms, such as malaria, dengue, brucellosis and leptospirosis, can be confused with typhoid in both adults and children.

A 2008 study reported on the incidence of typhoid fever detected through passive surveillance (and through modified passive surveillance in two countries where additional health clinics were introduced into the community) in five Asian countries *(25)*. The incidence of typhoid fever ranged from 15.3 per 100 000 person-years among people aged 5–60 years in China to 451.7 per 100 000 person-years among children aged 2–15 years in Pakistan *(25)*. More recently, its incidence in Nepal has been estimated to range from 297 to 449 per 100 000 person-years, with greater incidence occurring during the summer months *(26)*. Incidence data from the placebo control groups in vaccine trials also provide information on the incidence of typhoid fever in multiple geographical areas and locations.

However, because vaccine efficacy trials are typically carried out in areas with high endemicity, caution must be exercised when extrapolating these incidence rates to other populations. New data on age-specific occurrence in certain geographical regions, as in some sites in South Asia, confirm that typhoid fever of sufficient severity to seek medical care is common in the 1–4 year-old age group, with a large proportion of disease occurring in children between 6 months and 2 years of age *(17)*.

There has also been an increasing number of major outbreaks associated with antimicrobial-resistant *S.* Typhi *(17, 27–31)*, with the increased occurrence of outbreaks due to multidrug-resistant typhoidal *Salmonella* serovars being of particular concern. Extensively drug-resistant variants of *S.* Typhi have also emerged in India, Bangladesh and Pakistan that severely limit treatment options and are therefore becoming increasingly difficult to treat *(32, 33)*. The *S.* Typhi H58 clade, with IncHI1 plasmids that carry multidrug-resistance genes and target site mutations mediating fluoroquinolone resistance, is responsible for much of the recent and dramatic spread of resistant strains in countries, such as occurred in Pakistan in 2018 *(33, 34)*. This clade is believed to have emerged on the Indian subcontinent about 30 years ago and then spread to South-East Asia and more recently to sub-Saharan Africa *(33)*. The emergence of extensively antibiotic-resistant *S.* Typhi (resistant to first- and second-line antibiotics) and the implications of this for disease control were reviewed in 2017 *(20)*. The global pattern of drug-resistant *S.* Typhi is dynamic and changing in each location and over time. For example, in Ho Chi Minh City, Viet Nam the proportion of strains with a diminished susceptibility to fluoroquinolones increased from less than 5% to 80% within a few months in 1998 *(35)*. A large-scale outbreak of extensively drug-resistant typhoid in Pakistan further demonstrates the importance of understanding local resistance patterns to enable the selection of appropriate antibiotics for the management of typhoid fever cases *(33)*.

Prior to the availability of antibacterial agents, typhoid resulted in a case-fatality rate of approximately 10–20% *(36)*. Current estimates covering the post-antibiotic era range from 1% to 4% of those who receive adequate therapy *(37)*. Most of the mortality occurs in developing countries, predominantly in Asia. A 2008 review *(19)* reported community-based mortality ranging from 0% to 1.8% across five studies in developing countries; hospital-based mortality ranged from 0% to 13.9% across all ages in 12 studies; and in children younger than 15 years, mortality ranged from 0% to 14.8% across 13 studies. Hospitalization rates of 2–40% have also been reported *(25)* indicating that the disease can be severe in a considerable proportion of patients. The evolution and spread of multiple antibiotic resistant *S.* Typhi described above further complicates the situation and leads to an increased proportion of patients experiencing clinical treatment failure and complications, increasing hospital admission and prolonged hospital stay *(20)*.

Few studies have estimated the prevalence of chronic carriers of typhoid and paratyphoid in developing countries. One survey in Santiago, Chile, conducted when typhoid fever was highly endemic there in the 1970s, estimated a crude prevalence of 694 typhoid carriers per 100 000 population *(38)*. In Kathmandu, Nepal, among 404 patients (316 females and 88 males) with gall bladder disease undergoing cholecystectomy, *S.* Typhi was isolated from 3.0% of bile cultures and *S.* Paratyphi A from 2.2% *(39)*. Since the overall prevalence of cholelithiasis in the population of Kathmandu was not known, the overall prevalence of chronic carriage in that population could not be calculated. The role of chronic carriers in the transmission of typhoid fever is still unclear *(17)* but is thought to vary between settings of high, medium and low disease incidence *(18, 40)*. However, chronic carriers may represent a long-term reservoir of infection and contribute to the persistence of typhoid fever through ongoing shedding of *S.* Typhi and *S.* Paratyphi into the environment, possibly contaminating water supplies.

Clinical features

S. Typhi infection results in a wide spectrum of clinical features, most often characterized by persisting high-grade fever, abdominal discomfort, malaise and headache. Important clinical signs in hospitalized patients include hepatomegaly (41%), toxicity (33%), splenomegaly (20%), obtundation (2%) and ileus (1%) *(41)*. Before antibacterial agents became available, gross bleeding from the gastrointestinal tract and perforations occurred in 1–3% of untreated patients and hospital-based reports suggest that more than 50% of patients may have serious complications. In one 2005 study *(42)*, numerous extra-intestinal complications were reported on involving the central nervous system (3–55%), the hepatobiliary system (1–26%), the cardiovascular system (1–5%), the pulmonary system (1–6%), bones and joints (less than 1%) and the haematological system (rarely). Intestinal perforations leading to peritonitis and death continue to be reported, albeit rarely, in some settings. Interestingly, the emergence of multidrug-resistant strains has been associated not only with failure to respond to antibiotic treatment but also with changes in the severity and clinical profile of enteric fever *(5, 43)*.

Immune responses to natural infection

Natural typhoid infection is usually associated with detectable serum antibodies and mucosal secretory immunoglobulin A (IgA) intestinal antibody against various *S.* Typhi antigens. However, cell-mediated immune responses are also measurable *(44–48)*. In areas where typhoid is endemic, there is an age-related increase in the prevalence and geometric mean titre of anti-Vi antibodies *(49)*. Anti-flagella (H antigen) serum IgG antibodies following natural infection are long lived and have been studied for seroepidemiological surveys *(50)*.

While serological responses to LPS and flagella antigens tend to be quite strong and are commonly found in patients with culture-confirmed acute typhoid fever, only about 20% of such patients exhibit significant levels of anti-Vi antibody *(51, 52)*. In contrast, high concentrations of anti-Vi serum IgG antibody are detected in 80–90% of chronic carriers *(51, 52)*.

Cell-mediated immunity also appears to play a part in protection – it has been observed that peripheral blood mononuclear leukocytes of healthy adults residing in typhoid-endemic areas, and who have no history of typhoid, proliferate upon exposure to *S.* Typhi antigens *(53)*.

Disease control

As with other enteric and diarrhoeal diseases, typhoid fever occurs predominantly in populations with inadequate access to safe water and basic sanitation. Effective typhoid control requires a comprehensive approach that combines immediate measures, such as accurate and rapid diagnostic confirmation of infection and timely administration of appropriate antibiotic treatment, with sustainable longer-term solutions such as providing access to safe water and basic sanitation services, health education, appropriate hygiene among food handlers and typhoid vaccination. There is evidence that vaccination against typhoid can substantially reduce typhoid fever burden when targeted towards high-risk age groups and geographical areas, and when combined with improved sanitation *(54)*. The most recent WHO position paper on the use of typhoid vaccines was published in 2018 *(17)*.

Typhoid vaccines

Inactivated whole-cell vaccine

Inactivated *S.* Typhi bacteria (heat inactivated and phenol preserved) were first used to prepare parenteral vaccines more than 100 years ago. In the 1960s, WHO sponsored field trials that evaluated the efficacy of inactivated parenteral whole-cell vaccines in several countries *(55, 56)* and documented a moderate level of efficacy lasting up to 7 years *(57)*. Data from studies of human immune responses and immunogenicity studies in rabbits suggested that anti-H antibodies might represent an immune correlate of protection *(58)*; later extrapolation from the results of mouse protection studies suggested that responses to Vi antigen may also correlate with protection *(59, 60)*. However, these vaccines were associated with considerable rates of systemic adverse reactions *(61)* and never became widely accepted public health tools, and are thus no longer produced.

Live-attenuated Ty21a oral vaccine

In the early 1970s, an attenuated strain of *S.* Typhi was developed through chemically induced mutagenesis of pathogenic *S.* Typhi strain Ty2 *(59)*. The resultant mutant strain lost the activity of the epimerase enzyme encoded by the *galE* gene and no longer expressed the Vi antigen. The vaccine was found to be stable, safe and efficacious in adults as well as children *(62–66)*. The level of protective immunity achieved varied according to the formulation of the vaccine, the number of doses administered and the interval between doses. For example, three doses of a provisional formulation of vaccine administered to around 32 000 children (aged 6–7 years) in Alexandria, Egypt gave a point estimate of efficacy of 95% (95% confidence interval (CI) = 77–99%) during 3 years of follow-up *(67)*. Three doses of enteric-coated capsules administered to Chilean schoolchildren (aged 6–19 years) using two different dose intervals (either alternate days or 21 days between doses) gave a point estimate of efficacy of 67% (95% CI = 47–79%) during 3 years of follow-up. For the group receiving doses on alternate days, the point estimate of protection over 7 years was 62% (95% CI = 48–73%) *(56, 68)*. For the group receiving each dose after a 21-day interval, the corresponding point estimate of protection was 49% (95% CI = 24–66%). Another trial among Chilean schoolchildren involved the administration of four doses within 7 days and demonstrated even greater protection *(69)*. Human challenge studies showed that 5–8 doses of Ty21a oral vaccine resulted in high rates of anti-LPS antibody seroconversion and 87% protective efficacy *(70)*. However, more recent human challenge studies showed that a three-dose Ty21a immunization schedule resulted in a protective efficacy of only 35% after challenge when using the end-points of fever and/or bacteraemia as a diagnosis of typhoid *(71)*. When efficacy was recalculated using the same definition for typhoid diagnosis used in the original vaccine/challenge studies (fever with subsequent microbiological confirmation) then Ty21a efficacy reached 80% *(71)*, which is similar to that reported in the older challenge studies.

Two field trials in Chile *(66)* and Indonesia *(65)* compared the use of enteric-coated capsules with three doses of the liquid formulation. In both trials, the liquid formulation was associated with greater efficacy than the capsules. In Chile, where the doses were given on alternate days, the results for the liquid formulation were superior to those obtained in Indonesia, where the doses were administered 1 week apart (point estimate of efficacy = 77% in Chile and 53% in Indonesia). In Chile, 78% protection was documented up to 5 years following vaccination with the liquid formulation *(68)*. However, the previously marketed liquid formulation is no longer produced, and only enteric-coated capsules are currently available *(17)*.

All countries in which Ty21a is licensed utilize a three-dose course of enteric-coated capsules taken on alternate days, with the exception of the United States of America (USA) and Canada, which both use a four-dose course. This vaccine was first licensed in Europe in 1983 and in the USA in 1989, and is approved for use in individuals older than 6 years. Because the vaccine is highly acid labile, stomach acidity must be neutralized or bypassed when Ty21a is fed orally. There is indirect evidence that large-scale vaccination with Ty21a may provide some degree of protection against typhoid to people who have not been vaccinated through the mechanism of herd protection.

Vi polysaccharide vaccine (unconjugated)

Technological advances in the late 1960s made it possible to purify Vi polysaccharide without damaging its antigenic properties and to prepare vaccines that are almost totally free of contaminating LPS *(72)*; these vaccines are associated with low rates of febrile reactions (1–2%). Vi polysaccharide vaccine was first licensed in the USA in 1994 and since then several products have been licensed for use in individuals aged 2 years and older. One such product (Typhim-Vi) has been prequalified by WHO.[1]

The immunological basis of protection by purified Vi polysaccharide parenteral vaccines is the generation of serum anti-Vi IgG antibodies in 85–90% of vaccine recipients older than 2 years.

Clinical trials with these vaccines showed a rise in anti-Vi antibody titres in adults and children *(73–75)*. However, subsequent inoculations with Vi did not boost the antibody response. Although a single dose has been associated with the persistence of antibodies for up to 3 years in some recipients, many adult recipients in non-endemic areas showed a marked drop in antibody levels after 2 years *(76, 77)*. An outbreak of typhoid fever among French soldiers deployed in Côte d'Ivoire indicated that the risk of typhoid fever was significantly higher in those who had been vaccinated more than 3 years previously compared to those who had been vaccinated in the 3 years prior to the outbreak *(78)*.

Field trials in children and adults in Nepal given a single 25 µg dose showed 72% vaccine efficacy during 17 months of follow-up *(73)* and a field trial in schoolchildren in South Africa (also using a single 25 µg dose) showed 60% protection during 21 months of follow-up *(74)*. In South Africa, protection was found to decline to 55% at 3 years *(79)*. Another field trial in China in people aged 3–50 years given a single 30 µg dose showed 69% efficacy during 19 months of follow-up *(80)*. Thus, while a single dose of an unconjugated Vi polysaccharide vaccine provides moderate protection, the available data suggest that protective efficacy does not last beyond 3 years and revaccination is necessary within that time.

Most data suggest that children younger than 5 years respond poorly to unconjugated Vi polysaccharide vaccines *(81)*. However, one cluster-randomized trial in Kolkata, India *(82)* found that protective efficacy among young children (aged 2–4 years) was 80%, which was higher than that observed in children aged 5–14 years (56%) and in older persons (46%). In contrast, a cluster-randomized field trial of similar design and using the same Vi polysaccharide vaccine in Karachi, Pakistan reported an adjusted total protective effectiveness of −38% (95% CI = minus 192–35%) for children aged 2–5 years compared with 57% (95% CI = 6–81%) for children aged 5–16 years *(81)*.

Thus, a single dose of unconjugated Vi polysaccharide vaccine can provide moderate protection for a limited duration, but such vaccines have the usual limitations associated with polysaccharide vaccines, including poor immunogenicity in infants and young children,

[1] See: WHO Prequalified Vaccines at: https://extranet.who.int/gavi/PQ_Web/

short-lived immunity and lack of anamnestic immune responses to subsequent doses *(76, 82, 83)*.

Vi polysaccharide–protein conjugate vaccine

Experience with other polysaccharide–protein conjugate vaccines (such as *Haemophilus influenzae* type b, meningococcal and pneumococcal vaccines) has shown that conjugation to a carrier protein overcomes many of the limitations associated with unconjugated bacterial polysaccharides. On the basis of this, several Vi polysaccharide–protein conjugate vaccines have been developed or are under development. These include vaccines based on Vi polysaccharide conjugated to tetanus toxoid (TT), diphtheria toxoid (DT), the nontoxic mutant of diphtheria toxin cross-reactive material 197 (CRM_{197}) as well as on the prototype Vi polysaccharide conjugated to nontoxic recombinant exoprotein A of *Pseudomonas aeruginosa* (Vi-rEPA) *(84)*. One TCV that uses Vi prepared from *C. freundii* s.l. and CRM_{197} as the carrier protein has been shown to elicit a higher level of anti-Vi IgG compared to an unconjugated Vi polysaccharide vaccine in European adults who have never been exposed to typhoid fever *(85)*. Vi preparations from *C. freundii* s.l. are immunologically indistinguishable from and structurally similar to those from *S.* Typhi *(85–87)*, though size and viscosity differences have been observed for Vi polysaccharide from *S.* Typhi and *C. freundii* s.l. using high-performance size-exclusion chromatography (HPSEC) *(87)*.

Four TCVs have been licensed in India since 2008; three consisting of Vi polysaccharide conjugated to TT and one to CRM_{197}. Other TCVs are in late-stage development in some Asian countries. Typbar-TCV (a Vi–TT conjugate vaccine) was licensed in India in 2013 for use in children aged 6 months or older and in adults up to 45 years of age on the basis of immunogenicity and safety demonstrated in a Phase III study in an endemic setting *(17, 88)*. The results showed that anti-Vi antibody titres were significantly higher among recipients of Typbar-TCV than those vaccinated with the unconjugated Vi polysaccharide vaccine. Furthermore, the high geometric mean titres of IgG anti-Vi antibodies elicited by a single dose of Typbar-TCV persisted for up to 5 years in approximately 84% of children. The vaccine was prequalified by WHO in December 2017. A protective efficacy of 87.1% (95% CI = 47.2–96.9%) against persistent fever associated with positive blood culture for *S.* Typhi was subsequently demonstrated in human challenge studies *(89)*. Interim data on the efficacy of Typbar-TCV in an endemic population in Nepal have also recently been published *(26)*. These data, from a Phase III participant-observer blinded randomized study in children aged 9 months to 16 years of age, confirmed that a single dose of Typbar-TCV was safe, immunogenic and effective in this field setting, with an efficacy of 81.6% (95% CI = 58.8–91.8%) *(26)*. This conclusion is supported by new data from Pakistan (vaccine efficacy = ~89%) and India (vaccine efficacy = 82%). In view of the improved immunological properties of TCVs compared to the other available typhoid vaccine types, their suitability for use in young children and longer expected duration of protection, the WHO SAGE recommended such vaccines as the preferred type for use in individuals from 6 months to 45 years of age, and that the introduction of TCVs be prioritized in countries with the highest burden of typhoid fever or with a high burden of antimicrobial-resistant *S.* Typhi *(17)*.

Although no internationally agreed correlates or surrogates of protection have yet been identified for Vi conjugate vaccines, a number of suggested correlates have been proposed. Based on the assay used to measure anti-Vi IgG serum antibodies generated in response to the prototype United States National Institutes of Health Vi-rEPA conjugate vaccine in Viet Nam, a threshold value of 4.3 µg/mL anti-Vi antibody measured by enzyme-linked immunosorbent assay (ELISA) was found to be associated with a high level of sustained protection lasting 4 years after vaccination *(90, 91)*. A placebo-controlled

randomized double-blind study in Vietnamese children aged 2–5 years in a highly endemic area produced an estimated efficacy of 89% for the Vi-rEPA vaccine over 46 months of follow-up *(92, 93)*. However, although a study to evaluate Vi-TT in Nepal *(94)* found that higher anti-Vi IgG levels are associated with greater protection against typhoid infection, no threshold level could be identified at which the probability of infection becomes negligible within the range of antibody levels induced by vaccination.

It is acknowledged that there are difficulties in comparing any immunogenicity data generated using new TCVs and current ELISA protocols to the data generated in the original Vi-rEPA trial in Viet Nam *(20)*. However, the inclusion of a working reference serum calibrated against the First WHO International Standard for anti-typhoid capsular Vi polysaccharide immunoglobulin G (see **International reference materials** below) can improve the interpretation of data from clinical trials *(95, 96)*. The use of this WHO international standard will ensure consistency in determining serum titres and thus allow for the comparison of data generated by different assays and/or different laboratories.

It has been suggested that variability in the biophysical properties of antibodies induced by Vi polysaccharide and Vi-TT conjugated vaccines (such as antibody subclass distribution and avidity) may also impact protective outcomes. One recent study *(97)* identified serum Vi IgA as a biomarker of protective immunity against typhoid fever and quantified the concentration of Vi IgA in vaccine recipients. However, no correlate of protection was identified and it was concluded that further work was needed to determine whether IgA represents a true correlate of protection or a surrogate marker of another underlying immune response.

Challenge studies

The development of vaccines against typhoid fever has been complicated by the human host restriction of *S.* Typhi, the lack of clear correlates of protection, the required scale of field trials of efficacy and uncertainty about the estimation of vaccine impact. Historically, only the chimpanzee model of the 1960s demonstrated a pathogenesis and clinical illness somewhat resembling typhoid fever in humans *(98–101)*. However, the chimpanzee model is no longer permissible and recent animal models (including ones based on "humanized" small animals) have not been able to mimic the disease process of human typhoid, despite many attempts *(102–107)*. Instead, a human challenge model has been used to overcome some of these difficulties and to provide direct estimation of efficacy in vaccine recipients who are deliberately challenged with the pathogen in a controlled setting *(108, 109)*. The first setting during the 1950s to early 1970s involved volunteers in a penal institution *(70, 110–112)* whereas more recent studies have involved community volunteers *(108, 109)*.

International reference materials

Two WHO international standards for Vi polysaccharide have been developed to measure the polysaccharide content of typhoid vaccines *(113–115)*. The Vi polysaccharide content of these two standards was assessed using quantitative nuclear magnetic resonance (qNMR). The First WHO International Standard for *Salmonella* Typhi Vi polysaccharide (NIBSC code 16/126) has a content of 2.03 ± 0.10 mg Vi polysaccharide/ampoule. The First WHO International Standard for *Citrobacter freundii* Vi polysaccharide (NIBSC code 12/244) has a content of 1.94 ± 0.12 mg Vi polysaccharide/ampoule *(113–115)*. Both standards can be used in physicochemical assays, for example, high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) or in immunoassays such as rocket immunoelectrophoresis to measure the amount of Vi polysaccharide in final product, bulk

conjugate and process intermediates. In addition, these Vi polysaccharide standards can be used as coating antigens for in-house ELISAs *(115–117)*. When analyzing the content of Vi polysaccharide vaccines the homologous Vi polysaccharide standard should be used. For example, if the conjugate has been made with *Citrobacter* Vi polysaccharide then the *Citrobacter* Vi polysaccharide standard would be the more appropriate standard to use. The use of these WHO standards decreases the variability of in-house analytical assays *(114, 115)*.

In addition, a First WHO International Standard for anti-typhoid capsular Vi polysaccharide IgG (human) (NIBSC code 16/138) is available and consists of pooled post-immunization sera obtained following vaccination with plain Vi polysaccharide or conjugated Vi polysaccharide according to the immunization schedule of Jin et al. *(89)*. This international standard was evaluated in both commercial and in-house ELISAs, and assigned a concentration of 100 International Units (IU)/ampoule *(95, 116, 117)*. This primary reference standard should be used as a calibrant for in-house and working standards that are used to evaluate the immunogenicity of licensed vaccines and vaccine candidates in clinical studies *(116, 117)*. The American national reference reagent Vi-IgG$_{R1,2011}$, distributed by the United States Food and Drug Administration, was also included in the collaborative study to establish the standard material 16/138 and was determined to contain 163 IU/mL *(116)*. As Vi-IgG$_{R1,2011}$ had been established as containing 33 µg anti-Vi IgG/mL *(90)* (equivalent to 33 µg/vial) it can be assumed that 1 µg anti-Vi IgG/mL is equivalent to 4.94 IU/mL.

A further collaborative study was then conducted in which the standard materials 16/138 and 12/244 were used to evaluate a standardized in-house ELISA based on a co-coating of Vi polysaccharide and poly-L-lysine. The results obtained indicate that this generic assay would be a suitable alternative to the commercial Vi polysaccharide ELISA *(96)*.

All of the above WHO international standards are available from the National Institute for Biological Standards and Control, Potters Bar, the United Kingdom.[1] For the latest list of appropriate WHO international standards and reference materials, the WHO Catalogue of International Reference Preparations should be consulted.[2]

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International name and proper name

The international name of the vaccine should be “typhoid conjugate vaccine”. The proper name should be the equivalent of the international name in the language of the country in which the vaccine is licensed.

The use of the international name should be limited to vaccines that meet the specifications given below.

A.1.2 Descriptive definition

A typhoid conjugate vaccine (TCV) is a preparation of *S.* Typhi or *C. freundii* s.l. Vi polysaccharide covalently linked to a carrier protein. It may be formulated with a suitable adjuvant and/or a preservative. It should be presented as a sterile aqueous suspension or as freeze-dried material. The preparation should meet all of the specifications given below.

[1] See: www.nibsc.org/products
[2] See: www.who.int/bloodproducts/catalogue

A.2 General manufacturing recommendations

The general guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles *(118)* and WHO good manufacturing practices for biological products *(119)* should be followed at establishments manufacturing Vi conjugate vaccines.

The production method should be shown to consistently yield Vi conjugate vaccines of satisfactory quality as outlined in these WHO Recommendations. All assay procedures used for quality control of the conjugate vaccine and vaccine intermediates should be validated. Post-licensure changes to the manufacturing process and quality control methods should also be validated and may require approval from the NRA prior to implementation *(120–122).*

Production strains for Vi polysaccharide and the carrier proteins may represent a hazard to human health and should be handled under appropriate containment conditions based on risk assessment and applicable national and local regulations *(123).* Standard operating procedures should be developed to deal with emergencies arising from accidental spills, leaks or other accidents. Personnel employed by the production and control facilities should be adequately trained. Appropriate protective measures, including vaccination, should be implemented if available.

If raw materials used in the culture media or in subsequent manufacturing steps contain materials of animal origin, they should comply with the current *WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (124).*

A.3 Control of source materials

A.3.1 Bacterial strains

The bacterial strain used for preparing Vi polysaccharide or carrier protein should be from a single well-characterized stock that can be identified by a record of its history, including the source from which it was obtained, number of passages and the tests used to determine the characteristics of the strain. Information regarding materials of animal origin used during passages of the bacterial strain should be provided, such as compliance with the current *WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (124)* or a statement of risk assessment.

A.3.1.1 Bacterial strain for preparing Vi polysaccharide

The strain used should be capable of stably producing Vi polysaccharide. *S.* Typhi and *C. freundii* s.l. have been shown to be suitable sources of Vi polysaccharide. Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, immunochemical tests or any other suitable method may be used for confirming the identity of the polysaccharide.

A.3.2 Bacterial seed lot system

The production of both Vi polysaccharide and the carrier protein should be based on a seed lot system consisting of a master seed and a working seed. Cultures derived from the working seed should have the same characteristics as the cultures of the strain from which the master seed lot was derived. Each new seed lot prepared should be characterized using appropriate control tests to ensure comparable quality attributes to those of the previous seed lot. New

seed lots should also be shown to have comparable Vi polysaccharide or carrier protein yields in routine manufacturing prior to their use.

The control tests for master and working seed lots may include culture purity, strain identity, Vi polysaccharide immunoassay or any other method(s) suitable for the characterization of Vi polysaccharide or carrier protein.

A.3.3 Bacterial culture media

Manufacturers are encouraged to avoid the use of materials of animal origin. However, if the culture medium does contain materials of animal origin, these should comply with the current *WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (124)*. The use of materials of animal origin should be discussed with and approved by the NRA.

The culture medium used to prepare bacterial seed lots and commercial vaccine lots should also be free from substances likely to cause toxic or allergic reactions in humans. Additionally, the liquid culture medium used to produce polysaccharide intermediate should be free from ingredients that will form a precipitate upon addition of chemical compounds, such as hexadecyltrimethylammonium bromide (CTAB), used for the purification of the Vi polysaccharide.

A.4 Control of vaccine production

A.4.1 Control of polysaccharide antigen production

The Vi polysaccharides that are used in licensed vaccines are defined chemical substances if they are prepared to similar specifications, for example as described in the WHO Requirements for Vi polysaccharide typhoid vaccine *(125)* and the requirements set out in the following sections of the current document. As a result, it is expected that they will be suitable for the preparation of TCVs.

A.4.1.1 Single harvests for preparing Vi polysaccharide antigen

The consistency of the production process should be demonstrated by monitoring the growth of the organisms and the yield of Vi polysaccharide.

A.4.1.1.1 Consistency of microbial growth for antigen production

The consistency of the growth of production strains should be demonstrated by monitoring the growth rate, pH, pO_2 and the final yield of Vi polysaccharide – though monitoring should not be limited to these parameters.

A.4.1.1.2 Bacterial purity

Samples of the culture should be taken before inactivation and examined for microbial contamination. The purity of the culture should be verified using suitable methods, such as inoculation on appropriate culture media. If contamination is found, the culture and any product derived from it should be discarded.

A.4.1.2 Bacterial inactivation and antigen purification

Generally, the production culture is inactivated using a suitable method such as chemical treatment (for example, with formaldehyde), heating or other alternative methods prior to purification. The inactivation process should be validated and monitored using a validated

test during routine manufacturing. If a chemical agent is used for inactivation, its residual level should be controlled as described in section A.4.1.3.10 below.

After inactivation where appropriate, the biomass of *S.* Typhi or *C. freundii* s.l. is removed using an appropriate method such as centrifugation or tangential flow filtration. The Vi polysaccharide may be then purified from the supernatant by precipitation with CTAB or by other suitable methods approved by the NRA. Bioburden should be monitored during purification. The purified Vi polysaccharide should be stored under appropriate conditions that have been shown to retain the integrity of the Vi polysaccharide (for example, powder at 2–8 °C or lower and solution at −20 °C or lower). Hold times should be based on the results of stability studies and approved by the NRA.

A.4.1.3 Control of purified Vi polysaccharide antigen

Each lot of purified Vi polysaccharide should be tested for identity and purity, as well as the additional parameters described below. All tests should be validated and any test limits or ranges not defined by a pharmacopoeia should be agreed with the NRA.

A.4.1.3.1 Identity

Vi polysaccharide is a linear homopolymer composed of (l →4)-2-acetamido-2-deoxy-α-D-galacturonic acid that is *O*-acetylated at carbon-3 *(126)*.

A test should be performed on the purified polysaccharide to verify its identity, such as NMR spectroscopy *(127)* or a suitable immunoassay, as appropriate and convenient.

A.4.1.3.2 Molecular size or mass distribution

The molecular size or mass distribution of each lot of purified polysaccharide should be estimated to assess the consistency of each batch. The distribution constant (K_D) should be determined by measuring the molecular size distribution of the polysaccharide at the main peak of the elution curve obtained by a suitable chromatographic method. The K_D value or the mass distribution limits, or both, should be established and shown to be consistent from lot to lot for a given product. To ensure consistency and a defined proportion of high molecular size polysaccharide for gel filtration using HPSEC, typically at least 50% of the Vi polysaccharide should elute at a K_D value less than a predefined value, depending on the chromatographic method used. However, if molecular weight (MW) is determined by static light scattering then there is no need for a K_D value since it is a coefficient that is dependent on the column used. As an alternative, polysaccharide MW distribution can be determined by gel permeation chromatography using a MW standard calibration curve (that is, dextran, pullulans or polyethylene oxide standards) – the number average molecular weight (Mn), the weight average molecular weight (Mw) and the size average molecular weight (Mz) should be determined to describe the distribution.

An acceptable level of consistency should be agreed with the NRA. Alternatively, calculation of the peak width at the 50% level can be used to analyse the distribution of MW. Suitable detectors for this purpose include a refractive index detector *(128)*, alone or in combination with a static light scattering detector (for example, multi-angle laser light scattering detector) *(87)* and/or a viscometer. The methodology used should be validated to demonstrate sufficient resolution in the appropriate MW range. Manufacturers are encouraged to produce Vi polysaccharide that has a consistent distribution of molecular size.

Due to its high viscosity on molecular sizing columns, the Vi polysaccharide does not behave the same as other polysaccharides; therefore, column matrices and eluents should be carefully chosen to ensure a representative recovery *(87, 129)*.

A.4.1.3.3 Polysaccharide content

The concentration of the Vi polysaccharide in its *O*-acetylated acid form can be measured using NMR *(127)* or HPAEC-PAD *(131, 132)*, while methods such as the Hestrin method *(114, 130)* or the acridine orange method *(126, 131)* are also acceptable, and a suitable immunoassay, for example rocket immunoelectrophoresis or ELISA, may be considered. A suitable reference preparation of Vi polysaccharide calibrated against the First WHO International Standard for *Citrobacter freundii* Vi polysaccharide (NIBSC code 12/244) or against the First WHO International Standard for *Salmonella* Typhi Vi polysaccharide (NIBSC code 16/126) should be used where appropriate (see **International reference materials** above). These methods should be validated and agreed with the NRA.

A.4.1.3.4 O-acetyl content

The *O*-acetyl content of the purified Vi polysaccharide is important for the immunogenicity of Vi *(87, 126, 133)* and should be at least 2.0 mmol/g polysaccharide (52% *O*-acetylation), unless justified. The Hestrin method *(130)*, NMR *(127, 134)*, high-performance anion exchange chromatography with conductivity detection (HPAEC-CD) *(135)* or other suitable methods may be used to quantitatively determine *O*-acetylation. The methods and acceptance criteria used should be agreed with the NRA.

A.4.1.3.5 Moisture content

If the purified polysaccharide is to be stored as a dried form, the moisture content should be determined using suitable validated methods, and the results should be within established limits. The methods and acceptable limits used should be agreed with the NRA.

A.4.1.3.6 Protein impurity

The protein content should be determined using a suitable validated method, such as that of Lowry et al. *(136)*, and using bovine serum albumin as a reference. Sufficient polysaccharide should be assayed to accurately detect protein contamination. Each lot of purified polysaccharide should typically contain no more than 1% (weight/weight) of protein.

A.4.1.3.7 Nucleic acid impurity

Each lot of purified polysaccharide should contain no more than 2% nucleic acid by weight as determined by ultraviolet spectroscopy on the assumption that the absorbance of a 10 g/L nucleic acid solution contained in a cuvette of 1 cm path length at 260 nm is 200 *(137)*. Other validated methods may be used. Sufficient polysaccharide should be assayed to accurately determine nucleic acid contamination.

A.4.1.3.8 Phenol content

If phenol has been used to prepare the Vi polysaccharide antigen, each lot should be tested for phenol content using a validated method approved by the NRA. The phenol content should be expressed in µg/mg of purified Vi antigen and shown to be consistent and within the limits approved by the NRA.

A.4.1.3.9 Endotoxin

The endotoxin content of each lot of purified Vi polysaccharide should be determined and shown to be within limits agreed with the NRA. Suitable in vitro methods include the *Limulus* amoebocyte lysate (LAL) test.

A.4.1.3.10 Residual process-related contaminants

The levels of residual process-related contaminants in the purified polysaccharide (for example, CTAB, formaldehyde or other bacterial inactivating agent, and antifoaming agents) should be determined, and shown to be below the limits agreed with the NRA. The routine testing of each lot before release for residual process-related contaminants may be omitted once consistency of production has been demonstrated on a number of lots; this number should be agreed with the NRA.

A.4.1.4 Activated polysaccharide

Purified Vi polysaccharide is usually activated to enable conjugation; it may also be partially depolymerized or fragmented, either before or during the activation process.

A.4.1.4.1 Chemical activation

Several methods are satisfactory for the chemical activation modification of Vi polysaccharides prior to conjugation. The method that is chosen should be approved by the NRA. As part of the in-process control procedures, the processed Vi polysaccharide that will be used in the conjugation reaction should be assessed to determine the number of functional groups introduced.

A.4.1.4.2 Molecular size or mass distribution

If any size-reduction *(138, 139)* or activation steps are performed, the average size or mass distribution (that is, the degree of polymerization) of the processed Vi polysaccharide should be measured using a suitable method. The size or mass distribution should be controlled using appropriate limits as these may affect the reproducibility of the conjugation process as well as the immunogenicity of the conjugate.

A.4.2 Control of carrier protein production

A protein that is safe and, when covalently linked with polysaccharide, elicits a T-cell-dependent immune response against polysaccharide could potentially be used as a carrier protein. Suitable carrier proteins include, but are not limited to, TT, DT, CRM_{197} and rEPA.

A.4.2.1 Consistency of production of the carrier protein

The manufacturing process for a carrier protein should be shown to consistently yield batches that are suitable for the conjugation process. Adequate in-process control should be implemented to monitor critical process parameters, such as the growth rate of the microorganism, pH of production culture and the final yield of the carrier protein.

A.4.2.2 Characterization and purity of the carrier protein

Carrier proteins should be assayed for purity and concentration and tested to ensure they are nontoxic and appropriately immunogenic. All tests used to control the carrier protein should be approved by the NRA.

Preparations of TT and DT should satisfy the relevant WHO recommendations *(140, 141)*. CRM_{197} can be obtained from cultures of *Corynebacterium diphtheriae* C7/β197 *(142)* or by expression in other genetically modified microorganisms *(143)*. CRM_{197} with a purity of not less than 90% as determined by high-performance liquid chromatography (HPLC) should be prepared by column chromatographic methods. Residual host cell DNA content

should be determined and results should be within the limits approved by the NRA for the particular product. Testing for residual host cell DNA content may be omitted if adequate validation data are available. When CRM_{197} is produced in the same facility as DT, tests should be carried out to distinguish the CRM_{197} protein from the active toxin.

A test should be performed on the purified carrier protein to verify its identity. Mass spectrometry or a suitable immunoassay or physicochemical assay could be performed as appropriate and convenient.

Additionally, the carrier protein should be further characterized using appropriate physicochemical methods, such as: (a) sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE); (b) isoelectric focusing; (c) HPLC; (d) amino acid analysis; (e) amino acid sequencing; (f) circular dichroism; (g) fluorescence spectroscopy; (h) peptide mapping; or (i) mass spectrometry *(144)*. Outcomes should be within the specifications of the carrier protein that was used to prepare the TCV lots evaluated in the definitive clinical studies used for licensing.

A.4.2.3 Degree of activation of the carrier protein

Adipic acid dihydrazide (ADH) or other appropriate linkers, such as N-succinimidyl-3-(2-pyridyldithio)-propionate, can be used to modify the carrier protein. The level of protein modification should be monitored, quantified and be consistent. The use of an in-process control may be required. The reproducibility of the method used for modification should be validated.

The level of modification of the carrier protein by ADH can be assessed by determining the amount of hydrazide; this can be achieved by using colorimetric reactions with 2,4,6-trinitrobenzenesulfonic acid using ADH as a standard *(145–147)*. Other suitable methods include fluorescent tagging followed by HPLC or quadrupole time-of-flight mass spectrometry.

A.4.3 Conjugation and purification of the conjugate

A number of methods of conjugation are in use and all involve multistep processes *(131, 138, 145–147)*. Prior to demonstrating the immunogenicity of the Vi conjugate vaccine in clinical trials, both the method of conjugation and the control procedures should be established to ensure the reproducibility, stability and safety of the conjugate.

The derivatization and conjugation processes should be monitored and analyzed for unique reaction products. Residual unreacted functional groups or their derivatives are potentially capable of reacting in vivo and may be present following the conjugation process. The manufacturing process should be validated and the limits for unreacted activated functional groups (those that are known to be clinically relevant) at the conclusion of the conjugation process should be agreed with the NRA.

After the conjugate has been purified, the tests described below should be performed to assess the consistency of the production process. These tests are critical for ensuring consistency from lot to lot.

A.4.4 Control of the purified bulk conjugate

Tests for releasing purified bulk conjugate should be validated.

A.4.4.1 Identity

A suitable immunoassay or other method should be performed on the purified bulk conjugate to verify the identity of the Vi polysaccharide. Depending on the buffer used, NMR

spectroscopy may be used to confirm the identity and integrity of the polysaccharide in the purified bulk conjugate *(134, 148–150)*. The identity of the carrier protein should also be verified using an immunoassay or other suitable method.

A.4.4.2 Endotoxin

The endotoxin content of the purified bulk conjugate should be determined using a suitable in vitro method such as a LAL test and shown to be within limits agreed with the NRA.

A.4.4.3 O-acetyl content

The *O*-acetyl content of the purified bulk conjugate should be determined by NMR, Hestrin method or other appropriate methods. The specification for the *O*-acetyl content of the purified bulk conjugate should be agreed with the NRA. The specification for *O*-acetyl content of the conjugate bulk should not be higher than that set for the purified Vi polysaccharide.

A.4.4.4 Process- and product-related impurities

The purification procedures for the conjugate should remove any residual reagents that were used for conjugation and capping. The removal of reagents, their derivatives and reaction by-products such as ADH, phenol and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (known as EDC, EDAC or EDCI) should be confirmed using suitable tests or by validation of the purification process. The routine testing of each lot may be omitted once consistency of production has been demonstrated on a number of lots; this number should be agreed with the NRA.

The specifications of the residual reagents and the quantifiable methods to be used should be agreed upon in consultation with the NRA.

Process validation should also demonstrate that no significant covalent modification of the Vi polysaccharide itself has occurred, and the percentage of modified Vi monosaccharides should not exceed what was shown to be safe and immunogenic in clinical studies. An example of this is the use of EDC, which leads to N-acylurea modifications. The N-acylurea content can be readily measured using NMR.

A.4.4.5 Polysaccharide content

The content of Vi polysaccharide should be determined using an appropriate validated assay such as HPAEC-PAD *(90, 113, 131, 132)* or immunological methods (for example, rate nephelometry, rocket electrophoresis). For recommendations on suitable reference materials to use see **International reference materials** above.

A.4.4.6 Conjugated and unbound (free) polysaccharide

A limit for the presence of unbound (free) Vi polysaccharide relative to total Vi polysaccharide should be set for the purified bulk conjugate; this limit should be agreed with the NRA. Methods that have been used to assay unbound polysaccharide include size-exclusion chromatography–reverse phase liquid chromatography *(151)*, Capto Adhere anion-exchange resin binding *(129)* and deoxycholate precipitation *(152)* followed by HPAEC-PAD or other method listed in section A 4.4.5 above. Other suitable methods may be developed and validated.

A.4.4.7 Total protein and unbound (free) protein

The protein content of the purified bulk conjugate should be determined using an appropriate validated assay. Each batch should be tested for conjugated and unbound protein. The unbound protein content of the purified bulk conjugate should comply with the limit for the product that has been agreed with the NRA. Appropriate methods for determining unbound protein include HPLC and capillary electrophoresis.

A.4.4.8 Conjugation markers

The success of the conjugation process can be assessed by characterizing the conjugate using suitable methods. For example, an increase in the MW of the protein component of the conjugate compared with the carrier protein can be demonstrated using the Coomassie blue stain with SDS–PAGE; an increase in the MW of the conjugate compared with both the Vi polysaccharide and the protein components should be demonstrated by the gel filtration profile, HPSEC, capillary electrophoresis or other suitable method.

Where the chemistry of the conjugation reaction results in the creation of a unique linkage marker (for example, a unique amino acid, a linker or other measurable marker of conjugation) this should be quantified for each conjugate batch to assess the extent of the covalent reaction between the Vi polysaccharide and the carrier protein *(138)*. This assessment of the unique linkage marker may be omitted once the consistency of conjugation is established, with the agreement of the NRA.

A.4.4.9 Absence of reactive functional groups

The validation batches should be shown to be free of reactive functional groups or their derivatives that are suspected to be clinically relevant on the polysaccharide and the carrier protein.

Where possible, the presence of reactive functional groups (for example, those derived by ADH treatment) should be assessed for each batch. Alternatively, the product of the capping reaction may be monitored, or the capping reaction can be validated to show that reactive functional groups have been removed.

A.4.4.10 Ratio of polysaccharide to carrier protein

The ratio of polysaccharide to carrier protein (expressed, for example, as g/g or mol/mol) in the purified bulk conjugate should be calculated. For this ratio to be a suitable marker of conjugation, the content of each of the conjugate components prior to their use should be known. For each purified bulk conjugate, the ratio should be within the range approved by the NRA for that particular conjugate and should be consistent with the ratio in vaccine that has been shown to be effective in clinical trials.

A.4.4.11 Molecular size or mass distribution

It is important to evaluate the molecular size or mass of the polysaccharide–protein conjugate to establish the consistency of production, product homogeneity and stability during storage.

The relative molecular size of the polysaccharide–protein conjugate should be determined for each purified bulk conjugate using a gel matrix appropriate for the size of the conjugate *(87)*. The method used should be validated and should have the specificity required to distinguish the polysaccharide–protein conjugate from other components that may be present (for example, unbound protein or polysaccharide). The specification of molecular size or mass distribution should be vaccine specific and consistent with that of lots shown to be immunogenic in clinical trials.

Typically, the size of the polysaccharide–protein conjugate may be examined by methods such as gel filtration using HPSEC on an appropriate column. Since the ratio of polysaccharide to protein is an average value, characterization of this ratio over the molecular size or mass distribution (for example, by using dual monitoring of the column eluent) can provide further proof of the consistency of production *(144, 153).*

A.4.4.12 Bacterial and fungal sterility

The purified bulk conjugate should be tested for bacterial and fungal sterility according to the methods described in Part A, sections 5.1 and 5.2 of the WHO General requirements for the sterility of biological substances *(154),* or using methods approved by the NRA. If a preservative has been added then appropriate measures should be taken to prevent it from interfering with the tests.

A.4.4.13 Specific toxicity of the carrier protein

The purified bulk conjugate should be tested to confirm the absence of toxicity specific to the carrier protein where appropriate (for example, when DT or TT is used as the carrier protein). Alternatively, the absence of specific toxicity of the carrier protein may be demonstrated at the purified carrier protein stage if agreed with the NRA.

A.4.4.14 pH

The pH of each batch should be tested, and the results should be within the established range and compatible with stability data.

A.4.4.15 Appearance

The appearance of the purified bulk conjugate solution, with respect to its form and colour, should be examined by a suitable method and should meet the established specifications. For a Vi polysaccharide conjugated to a toxoid, the appearance is typically clear to moderately turbid, and colourless to pale yellow.

For a dried or lyophilized preparation, the appearance should be checked after reconstitution with the appropriate diluent and should meet the established specifications.

A.4.5 Preparation and control of the final bulk

A.4.5.1 Preparation

The final bulk is prepared by mixing a suitable quantity of the purified bulk conjugate with all the other vaccine constituents, which may include stabilizer, preservative and/or adjuvant. The final bulk should be prepared using a validated process and should meet the specifications based on the quality attributes of vaccine lots that have been shown to be safe and efficacious in clinical trials. If an adjuvant is used, it should be mixed with the final bulk at this stage. The use of a preservative in either single-dose or multi-dose vaccine vials is optional. If a preservative has been added, its effect on antigenicity and immunogenicity must be assessed to ensure that the preservative does not affect the immune response.

A.4.5.2 Test for bacterial and fungal sterility

Each final bulk should be tested for bacterial and fungal sterility according to the methods described in Part A, sections 5.1 and 5.2 of the WHO General requirements for the sterility of biological substances *(154),* or using methods approved by the NRA. If a preservative has

been added to the final bulk, appropriate measures should be taken to prevent it from interfering with the tests.

A.5 Filling and containers

The relevant guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles *(118)* and WHO good manufacturing practices for biological products *(119)* should be followed.

A.6 Control of the final product

A.6.1 Inspection of the final containers

All filled final containers should be inspected as part of the routine manufacturing process. Those containers showing abnormalities – such as vial defects, improper sealing, clumping or the presence of endogenous or exogenous particles – should be discarded. The test should be performed against a black, and a white, background, and according to pharmacopoeial specifications.

A.6.2 Control tests on the final lot

The following tests should be performed on each final lot of vaccine (that is, in the final container) and the tests used should be validated and approved by the NRA. The permissible limits for tests listed under this section should be justified and approved by the NRA.

A.6.2.1 Appearance

The appearance of the final container and its contents should be verified using a suitable method and should meet the established criteria with respect to form and colour. For freeze-dried vaccines, their appearance should be verified before and after reconstitution, and should meet the established criteria.

A.6.2.2 Identity

Identity tests on the Vi polysaccharide and the carrier protein should be performed on each final lot. An immunological test or a physicochemical assay may be used for the Vi polysaccharide and the carrier protein.

A.6.2.3 Bacterial and fungal sterility

The contents of the final containers should be tested for bacterial and fungal sterility according to the methods described in Part A, sections 5.1 and 5.2 of the WHO General requirements for the sterility of biological substances *(154)*, or using a method approved by the NRA.

A.6.2.4 Polysaccharide content

The amount of Vi polysaccharide conjugate in the final containers should be determined and shown to be within the specifications agreed with the NRA.

The formulations of conjugate vaccines produced by different manufacturers may differ. A quantitative assay for the Vi polysaccharide should be carried out. The specification should be justified based on the clinical lots shown to be safe and immunogenic, and approved by the NRA. Examples of tests that may be used include: (a) colorimetric methods;

(b) chromatographic methods (including HPLC and HPAEC-PAD); and (c) immunological methods (including rate nephelometry and rocket immunoelectrophoresis) as discussed in sections A.4.1.3.3 and A.4.4.5 of these Recommendations.

A.6.2.5 Unbound (free) polysaccharide

A limit for free Vi polysaccharide content should be set for each conjugate vaccine as discussed in section A.4.4.6 above. Assessing the level of unconjugated polysaccharide in the final lot may be technically demanding *(129)*; as an alternative, the molecular size of the conjugate could be determined for the final lot to confirm the integrity of the conjugate. A more-quantitative assessment of free Vi in solution can be performed by HPAEC-PAD following separation of the intact conjugate. An acceptable value should be consistent with the value seen in batches used for clinical trials that showed adequate immunogenicity and should be approved by the NRA.

A.6.2.6 O-acetyl content

The *O*-acetyl content of the Vi polysaccharide conjugate in the final container should be determined for each final lot by NMR *(127)* or by other appropriate methods, such as the Hestrin method *(130)*. Routine release testing of each lot for *O*-acetyl content in the final product may be omitted if:

- the NRA agrees; and
- the *O*-acetyl content is measured at the level of conjugate bulk; and
- process-validation data obtained during the product's development confirm that formulation and filling do not alter the integrity of the functional groups.

The specification for the *O*-acetyl content of the final lot should not be higher than that set for the conjugate bulk. A limit for the *O*-acetyl content of the Vi polysaccharide conjugate should be approved by the NRA *(133)*.

A.6.2.7 Molecular size or mass distribution

The molecular size or mass distribution of the polysaccharide–protein conjugate should be determined for each final lot using a gel matrix appropriate for the size of the conjugate. The analysis of molecular size or mass distribution for each final lot may be omitted provided that the NRA agrees and the test has been performed on the conjugate bulk (see section A.4.4.11 above).

A.6.2.8 Endotoxin or pyrogen content

The endotoxin content of the final product should be determined using a suitable in vitro assay such as a LAL test. The endotoxin content should be consistent with levels found to be acceptable in vaccine lots used in clinical trials and within the limits agreed with the NRA.

The need for pyrogenicity testing should be determined during the manufacturing development process. It should also be evaluated following any changes in the production process or relevant reported production inconsistencies that could influence the quality of the product with regard to its pyrogenicity. When required, the monocyte activation test (MAT) or rabbit pyrogenicity test may be used for monitoring potential pyrogenic activity subject to the agreement of the NRA.

A.6.2.9 Adjuvant content and degree of adsorption

If an adjuvant has been added to the vaccine, its content should be determined using a method approved by the NRA. The amount of the adjuvant should also be agreed with the NRA. If aluminium compounds are used as adjuvants, the aluminium content should not exceed 1.25 mg per single human dose.

The consistency of adsorption of the antigen to the adjuvant is important; the degree of adsorption should be tested in each final lot and should be within the range of values measured in vaccine lots shown to be clinically effective. The methods and specifications used should be approved by the NRA.

A.6.2.10 Preservative content

If a preservative has been added to the vaccine, its content should be determined using a method approved by the NRA.

The amount of preservative in each dose of the vaccine should be shown not to have any deleterious effect on the antigen or to impair the safety of the product in humans. The effectiveness of the preservative should be demonstrated, and the concentration used should be approved by the NRA.

A.6.2.11 pH

If the vaccine is a liquid preparation, the pH of each final lot should be tested, and the results should be within the range of values approved by the NRA. For a lyophilized preparation, the pH should also be measured after reconstitution with the appropriate diluent.

A.6.2.12 Moisture content

If the vaccine is a lyophilized preparation, the level of residual moisture should be determined, and the results should be within the limit agreed with the NRA.

A.6.2.13 Osmolality

The osmolality of the final lots should be determined and shown to be within the range considered to be safe for intramuscular administration to humans and agreed with the NRA. The test for osmolality may be omitted once consistency of production is demonstrated or justification is provided, with the agreement of the NRA.

A.6.2.14 Protein content

The protein content should be determined using an appropriate and validated assay. Routine release testing of each lot for protein content in the final product may be omitted if the NRA agrees.

A.6.3 Control of diluents

The general guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles *(118)* should be followed during the manufacture and quality control of the diluents used to reconstitute TCVs. An expiry date should be established for the diluents based upon stability data. For lot release of the diluent, tests should be done to assess its appearance, identity, volume and sterility, and the concentrations of its key components.

A.7 Records

The relevant guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles *(118)* and WHO good manufacturing practices for biological products *(119)* should be followed as appropriate for the level of development of the vaccine.

A.8 Retained samples

A sufficient number of lot samples of the product should be retained for future studies and needs. Vaccine lots that are to be used for clinical trials may serve as reference materials in the future and a sufficient number of vials should be reserved and stored appropriately for that purpose.

A.9 Labelling

The guidance on labelling provided in WHO good manufacturing practices for pharmaceutical products: main principles *(118)* and WHO good manufacturing practices for biological products *(119)* should be followed as appropriate and the label on the cartons enclosing one or more final containers, or the leaflet accompanying each container, should include:

- a statement that the vaccine fulfils Part A of these WHO Recommendations;
- the instruction that any vaccine in a lyophilized form should be used immediately after reconstitution; if data have been provided to the licensing authority indicating that the reconstituted vaccine may be stored for a limited time then the length of time should be specified;
- where needed, information on the volume and nature of the diluent to be added to reconstitute the lyophilized vaccine; this information should specify that the diluent approved by the NRA should be supplied by the vaccine manufacturer; and
- for multi-dose vials, the storage conditions and shelf-life after opening.

A.10 Distribution and transport

The guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles *(118)* and WHO good manufacturing practices for biological products *(119)* should be followed.

Shipments should be maintained within specified temperature ranges, and packages should contain cold-chain monitors. Further guidance on these and related issues is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products *(155)*.

A.11 Stability testing, storage and expiry date

The relevant guidance provided in WHO good manufacturing practices for biological products *(119)* should be followed. Any statements concerning storage temperature and expiry date that appear on primary or secondary packaging should be based on experimental evidence and should be approved by the NRA.

A.11.1 Stability testing

Adequate stability studies form an essential part of vaccine development. These studies should follow the general principles outlined in the WHO Guidelines on stability evaluation of vaccines *(156)* and WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions *(157)*. The shelf-life of the final product and the hold time of each process intermediate (such as the purified polysaccharide, the carrier protein and the purified bulk conjugate) should be established based on the results of real-time, real-condition stability studies, and approved by the NRA.

The stability of the vaccine in its final container and at the recommended storage temperature should be demonstrated to the satisfaction of the NRA on at least three lots of the final product manufactured from different bulk conjugates. In addition, a real-time real-condition stability study should be conducted on at least one final container lot produced each year.

A protocol should be established and followed for each stability study which specifies the stability-indicating parameters to be monitored, as well as the applicable specifications. Some stability-indicating parameters may change over the shelf-life as discussed below. The specifications should take into consideration the expected quality of the vaccine at the end of shelf-life and should be linked to lots demonstrated to be safe and effective/immunogenic in clinical trials. For vaccines intended for use under extended controlled temperature conditions, the manufacturer should refer to the WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions *(157)*.

The polysaccharide component of conjugate vaccines may be subject to gradual hydrolysis at a rate that may vary depending upon the type of conjugate, the formulation or adjuvant, the excipient and the conditions of storage. The hydrolysis may result in reduced molecular size of the Vi polysaccharide component, a reduction in *O*-acetyl content, a reduction in the amount of polysaccharide bound to the carrier protein, a change in pH, reduced molecular size of the conjugate, or some combination of these.

If applicable, the residual moisture should be monitored as part of stability testing and release testing.

Where applicable, the level of adsorption of the conjugate to the adjuvant should be shown to be within the limits agreed with the NRA, unless data show that the immunogenicity of the final product does not depend on the adsorption of the antigen to the adjuvant.

Accelerated stability studies may provide additional supporting evidence of the stability of the product or other product characteristics, or both, but are not recommended for establishing the shelf-life of the vaccine under a defined storage condition.

When any changes are made in the production process that may affect the stability of the product, the vaccine produced by the new method should be shown to be stable.

A.11.2 Storage conditions

The recommended long-term storage conditions should be based on the findings of the stability studies and should ensure that all stability-indicating parameters of the conjugate vaccine (for example, free saccharide) meet the required specifications at the end of the shelf-life. The labelled and packaged vaccine products should be stored at the recommended long-term storage conditions.

If approved by the NRA, the use of a vaccine under extended controlled temperature conditions requires specific monitoring as described in the WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions *(157)*.

A.11.3 Expiry date

The expiry date should be based on the shelf-life as supported by stability studies and approved by the NRA. The start of the dating period (for example, the date of formulation of final bulk or the date of filling) should be agreed with the NRA. The expiry dates for vaccine and diluent may be different from one another. If the vaccine and diluent are packaged together, the expiry date for the package should be that of the component with the earliest expiry date.

A.11.4 Expiry of reconstituted vaccine (if applicable)

For single-dose containers the reconstituted vaccine should be used immediately. For multi-dose containers the use of the reconstituted container should follow the WHO multi-dose vial policy[1], and this should be reflected in the package insert and supplied instructions.

Part B. Nonclinical evaluation of typhoid conjugate vaccines

B.1 General principles

Detailed WHO guidelines on the design, conduct, analysis and evaluation of nonclinical studies of vaccines are available separately *(158)* and should be read in conjunction with Part B of these WHO Recommendations. Plans for nonclinical studies of candidate vaccines should be discussed with the NRA prior to the review process.

B.2 Product characterization and process development

It is critical that vaccine production processes are appropriately standardized and controlled to ensure consistency in manufacturing and the collection of nonclinical data that may suggest safety and efficacy in humans.

Candidate formulations of Vi conjugate vaccines should be characterized to define the critical structural and chemical attributes that indicate that the polysaccharide, the conjugating protein and the conjugate product are sufficiently pure and stable, and their properties are consistent. The extent of product characterization may vary depending on the stage of vaccine development. Vaccine lots used in nonclinical studies should be adequately representative of those intended for use in clinical investigations. Ideally, the lots used should be the same as those used in the clinical studies. If this is not feasible then the lots should be comparable with respect to physicochemical data, stability and formulation.

B.3 Nonclinical immunogenicity studies

The immunogenicity of glycoconjugate vaccines can vary greatly between different animal species and between strains within a species. Therefore, animal models used for immunogenicity studies during glycoconjugate vaccine development programmes should be selected with care as they may be poorly predictive of efficacy in humans. Animal studies should only be conducted when they provide proof-of-concept information in support of a clinical development plan, and any animal testing plan used in vaccine development should incorporate 3Rs (Replace, Reduce, Refine) best practices.

[1] See: https://apps.who.int/iris/bitstream/handle/10665/135972/WHO_IVB_14.07_eng.pdf;sequence=1

Immunogenicity data derived from appropriately selected animal models may be useful in establishing the immunological characteristics of the Vi polysaccharide conjugate product, and may guide the selection of doses, schedules and routes of administration to be evaluated in clinical trials. When animal models are used for the preclinical testing of vaccine immunogenicity, they should elicit an anti-Vi IgG response that is significantly greater than that of the control group (for example, a group that receives unconjugated Vi polysaccharide vaccine). It should be noted that a booster response may not be observed following a second dose if the priming dose induced a maximal response. Therefore, a good understanding of the dose-immunogenic response should be established prior to evaluating any booster effect.

Immunogenicity studies have demonstrated that Vi polysaccharide conjugates induce anti-Vi IgG in mice *(102, 131, 138, 159–161)*. In humans, high levels of anti-Vi IgG are associated with greater levels of protection against typhoid infection *(90, 91, 94)* – although there is no agreement that this may be considered a true correlate or surrogate of protection. Based on these observations, the level of anti-Vi IgG elicited in mice may be considered as a primary end-point for nonclinical studies of the immunogenicity of Vi conjugate vaccines.

Nonclinical studies of immunogenicity may include an evaluation of seroconversion rates or geometric mean antibody titres, or both. When possible, nonclinical studies should be designed to assess relevant immune responses, including functional immune response (for example, by evaluating serum bactericidal antibodies, opsonophagocytic activity and serum-dependent opsonophagocytic killing). These studies may also address the interference that can occur among antigens when multi-antigen vaccines are used. In such cases, the response to each antigen should be evaluated.

Although there have been advances in the use of animal models, no ideal animal model exists that establishes direct serological or immunological correlates of clinical protection. In the absence of such a model, it is important to ensure consistency of production using modern physical, chemical and immuno-based quality control methods as described in Part A of these WHO Recommendations. Additionally, any changes in critical quality attributes should be assessed for their impact on immunogenicity. Once the physicochemical tests are validated, these non-animal methods are considered more appropriate for use in lot release processes than animal models.

B.4 Nonclinical toxicity and safety studies

The WHO guidelines on nonclinical evaluation of vaccines *(158)* should be followed when assessing toxicity and safety in an appropriate animal model. These studies should entail careful analysis of all major organs, as well as of tissues proximal to and distal from the site of administration, to detect unanticipated direct toxic effects. If the target population for the vaccine includes pregnant women, or women of childbearing age, developmental toxicity studies should also be considered unless there is a scientific and clinically sound justification showing that conducting such studies is unnecessary *(158)*.

Dose-response studies may not be necessary as the nonclinical evaluation of potential toxicity can be performed at a dose that maximizes both the exposure of the animal and the subsequent immune response (such as antibody titre). This dose can be determined during pilot dose-response and/or immunogenicity studies. The dosing frequency should be the same as, or greater than, the number of administrations intended in clinical studies, but the interval between doses should not be longer *(158)*.

Requirements for the toxicity testing of individual vaccine components or any novel proteins may vary between regulatory jurisdictions. These requirements should be scientifically justified as individual vaccine components may have different toxicity and safety profiles when present in the formulated product. Therefore, manufacturers are

encouraged to discuss these testing requirements with the NRA prior to commencing nonclinical studies.

Nonclinical safety studies should be conducted in accordance with the good laboratory practices described elsewhere *(162, 163)*. For ethical reasons, it is desirable to apply the 3Rs concept of "Replace, Reduce, Refine" to minimize the use of animals where scientifically appropriate.

Part C. Clinical evaluation of typhoid conjugate vaccines

C.1 General considerations

The general principles described in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations *(2)* apply to Vi conjugate vaccines and should be followed. In addition, a number of issues specific to the clinical development programme for Vi conjugate vaccines are discussed below. The WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products *(164)* are also available and should be consulted.

Vi conjugate vaccines have now been licensed in some countries for use in children aged 6 months or older and in adults up to 45 years of age, with one such vaccine having been prequalified by WHO *(17)*. The licensure of effective Vi conjugate vaccines in some countries and their availability through the WHO prequalification programme have implications for the pathway to approval and design of clinical studies in children above the age of 6 months and adults up to 45 years old. Information supporting the safety, immunogenicity, efficacy and effectiveness of Vi conjugate vaccines in typhoid endemic regions, as well as insights into putative immune correlates of protection, are continually emerging *(26, 88, 165–168)*. The principles for clinical evaluation outlined below are based on the current situation and should be read in light of the specific circumstances in the jurisdiction of the individual NRA.

C.2 Outline of the clinical development programme

It is recommended that the major part of the pre-licensure clinical development programme is conducted in subjects who are representative of the intended target population.

C.2.1 Dose and schedule

The early clinical development programme should provide a preliminary assessment of safety and should be suitable for identifying an appropriate dose of conjugated Vi antigen and dose regimen(s) for the target age group(s). Such studies are necessary for each candidate Vi conjugate vaccine that is developed since it is not possible to extrapolate the dose and schedule identified for one conjugate vaccine to another. This consideration applies even if the same carrier protein is used for two different Vi conjugate vaccines as experience with other conjugated polysaccharide vaccines has indicated that differences in conjugation chemistry can affect immune responses to the polysaccharide(s).

C.2.2 Demonstrating or inferring vaccine efficacy

In the absence of a pre-licensure efficacy study, pathways to approval of a candidate Vi conjugate vaccine in the jurisdiction of any one NRA may depend on the following:

- If there is a licensed Vi conjugate vaccine for which protective efficacy has been documented (the data may come from pre- and/or post-licensure efficacy studies

and/or from post-licensure studies of effectiveness), and subject to any pertinent national legislation, the efficacy of a candidate Vi conjugate vaccine may be inferred based on adequately designed comparative immunogenicity studies to bridge to the efficacy data for the licensed vaccine.

- If there are data that point to a specific anti-Vi antibody concentration that strongly correlates with efficacy, the efficacy of a candidate Vi conjugate vaccine may be inferred by estimating the proportion of baseline seronegative subjects with post-vaccination immune responses that exceed the concentration identified. In this situation, it may still be appropriate for an NRA to request that the sponsor compares the immune response to the candidate vaccine with the immune response to a licensed Vi conjugate vaccine for which protective efficacy has been demonstrated.
- If there is no widely accepted antibody concentration that strongly correlates with efficacy and no licensed Vi conjugate vaccine for which protective efficacy has been documented, it may be appropriate to infer the efficacy of a candidate Vi conjugate vaccine by comparing the immune response with a licensed unconjugated Vi polysaccharide vaccine in subjects aged 2 years and above. For further details see section C.4 below.

C.3 Assessment of the immune response

C.3.1 Immune parameters of interest

There are no well-established or standardized assays for assessing functional antibody responses to Vi-containing vaccines, and it is not known how the results of such assays correlate with vaccine efficacy.

Assessment of the immune response to licensed unconjugated *(82, 169, 170)* and conjugated *(26, 89, 90, 171)* Vi polysaccharide vaccines has predominantly relied upon ELISA methods to measure total anti-Vi IgG in serum. For unconjugated Vi polysaccharide vaccines, approval has often been based on directly comparing the proportion of subjects that achieves anti-Vi IgG of at least 1 µg/mL and/or the proportion that achieves at least a 4-fold increase in anti-Vi IgG from pre- to post-vaccination. A regional or in-house working reference serum preparation calibrated against the First WHO International Standard for anti-typhoid capsular Vi polysaccharide IgG (human) (see **International reference materials** above) should be used in the interpretation of immunogenicity data from clinical trials. The use of this WHO international standard improves consistency in the determination of serum titres and provides a basis for the comparison of data generated by different assays and/or different laboratories.

At present, there is no established or widely agreed immune correlate of protection for typhoid vaccines – though correlations between total serum antibody *(79)*, total anti-Vi IgG *(81, 92, 93, 172, 173)* or anti-Vi IgA *(97)* in serum and protection against typhoid have been described. A putative immune correlate of protection based on anti-Vi IgG has been proposed based on long-term follow-up of Vietnamese children who received a candidate Vi conjugate vaccine in a large efficacy trial. However, the value reported is specific to the assay that was applied during that study and it is not yet clear what the corresponding values may be when using alternative assays.

C.3.2 Considerations regarding the carrier protein

Proteins such as CRM_{197}, DT, TT and rEPA have been used in the production of various Vi conjugate vaccines. Based on experience with other types of conjugate vaccines that use

CRM_{197}, DT or TT as the carrier protein, there is a possibility that the immune response to the Vi conjugated antigen may be reduced or enhanced in subjects who have pre-existing high levels of tetanus or diphtheria antitoxin before vaccination. This phenomenon should be explored during the development of Vi conjugate vaccines; this may be accomplished by analysing post-vaccination responses and comparing these with pre-vaccination antibody concentrations to the protein carrier. The potential clinical significance of any effect requires careful consideration.

Depending on the target age range, it may be important to assess the effects of co-administering Vi conjugate vaccines with other routine vaccinations. Guidance on such studies, including instances in which co-administered vaccines contain the carrier protein, may be found in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations *(2)*.

C.3.3 Immune memory

Vi conjugate vaccines are expected to elicit T-cell-dependent immune responses, which can be assessed by administration of a post-priming Vi conjugate dose after an interval of at least 6–12 months. Details of the clinical assessment of priming may be found in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations *(2)*. Whether or not booster doses will be needed to maintain protection after successful priming with Vi conjugate vaccines is not yet known. Until this issue is resolved, plans should be put in place to document antibody persistence and to evaluate vaccine effectiveness.

C.4 Immunogenicity

This section should be read in conjunction with the guidance on comparative immunogenicity trials provided in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations *(2)*. The selection of the most appropriate licensed vaccine for use as a comparator in clinical studies must be agreed between the sponsor and the NRA.

C.4.1 Studies that compare conjugated Vi polysaccharide vaccines

If the aim of the study is to immunobridge efficacy documented with a licensed Vi conjugate vaccine to a candidate vaccine, the study should be designed to demonstrate the non-inferiority of the immune response elicited by the candidate vaccine when compared with a licensed Vi conjugate vaccine. The primary immune parameter for the purposes of immunobridging and the acceptance criteria for concluding that the candidate vaccine will have at least similar efficacy to the licensed vaccine should be predefined and agreed between the sponsor and the NRA.

If efficacy data have supported the derivation of an immune correlate of protection, the proportion of subjects that achieves at least this concentration after vaccination with the candidate vaccine should be the primary immune parameter. In this case, a direct comparison with a licensed Vi conjugate vaccine would not be essential though some NRAs may request that a comparison is made with a licensed Vi conjugate vaccine for which vaccine efficacy has been documented to provide a comparison of safety.

If the sponsor wishes, or is requested, to conduct a comparative study against a licensed Vi conjugate vaccine for which efficacy is not documented then demonstrating non-inferiority for the candidate versus licensed vaccine does not evidence the potential efficacy of the candidate vaccine. Therefore, either the immune responses to the candidate vaccine should be interpreted against an immune correlate of protection or threshold value or, if neither exists, consideration should be given to alternative study designs as described below.

C.4.2 Studies that compare Vi conjugate vaccines with unconjugated Vi polysaccharide vaccines

Studies that compare candidate Vi conjugate vaccines with licensed unconjugated Vi polysaccharide vaccines should only be conducted in subjects who are at least 2 years of age. It is recommended that such studies are conducted only if a licensed Vi conjugate vaccine comparator is not available and it is considered important to generate comparative safety and immunogenicity data versus a licensed vaccine (see section C.2 above). If such studies are to be the basis for approval, data should be generated for the age range for which a claim for use of the candidate vaccine will be sought. Studies should stratify subjects by appropriate age subgroups, or separate studies should be conducted in different age groups.

For potential approaches to the primary comparison of immune responses see section C.3 above.

The immune responses should be measured in samples collected at day 28 after the initial vaccination series has been completed (that is, after a single dose or after the last assigned dose of the primary series) or in samples collected at an alternative time point if this is justified by data on antibody kinetics.

C.4.3 Studies that compare a group vaccinated with a Vi conjugate vaccine with a control group that does not receive a vaccine containing Vi

There are two situations in which such studies may be considered:

- As explained in section C.2 above, if there is an established immune correlate of protection, a direct comparison of immune responses with a licensed vaccine is not necessary. However, such a comparison may still be useful for interpreting the safety data and for putting the immune responses to the candidate vaccine into context.
- In the absence of an immune correlate of protection or the possibility of immunobridging the candidate Vi conjugate vaccine to the protective efficacy of a licensed Vi conjugate vaccine, a study that compares a candidate Vi conjugate vaccine with an unvaccinated group could be considered for subjects under 2 years of age. A comparison between a candidate Vi conjugate vaccine and a licensed unconjugated Vi polysaccharide vaccine would not be appropriate due to lack of reliable protective immune responses to the latter in children under 2 years of age. In this situation, it is recommended that studies are based upon randomized allocation to the candidate Vi conjugate vaccine (that is, the vaccinated group) or to a licensed non-typhoid vaccine from which study subjects may derive some benefit (that is, the control group). To put the immune responses observed into context, the anti-Vi titres elicited by the candidate Vi conjugate vaccine in children under 2 years of age may be compared (either directly or in a cross-study comparison) with one or both of:

  - the immune response to an unconjugated Vi polysaccharide vaccine in subjects ≥ 2 years of age;
  - the immune response to the candidate Vi conjugate vaccine in subjects ≥ 2 years of age.

C.5 Efficacy

This section should be read in conjunction with the guidance on efficacy trials and effectiveness studies provided in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations *(2)*.

Protective efficacy studies against typhoid can be conducted only in endemic areas with relatively high rates of disease. If a protective efficacy study is conducted, it should compare the rates of febrile illnesses associated with a positive blood culture for *S.* Typhi between a group that receives the candidate Vi conjugate vaccine and an appropriate control group.

Successful typhoid challenge studies conducted in healthy adults using an appropriate and validated model (that is, one in which some protective efficacy of unconjugated Vi polysaccharide vaccines is detectable) could provide considerable supporting evidence of the efficacy of a Vi conjugate vaccine. Human challenge studies may provide information on the relationship between the immune response and various efficacy parameters. If, in consultation with the NRA, sponsors decide to conduct typhoid challenge studies in humans, they should be undertaken only by physicians with appropriate expertise, and in a carefully controlled setting, to ensure the safety of the volunteers *(109)*. Healthy adults that are expected or known to be naive to typhoid and typhoid vaccines should be screened to detect any underlying pre-existing conditions that could impact on safety. In particular, subjects who might be at risk of complications of typhoid should be excluded, including any subject with gall bladder disease. The challenge strain should be well characterized and there should be complete information available on its susceptibility to antibacterial agents.

An issue to consider after initial licensure is the possibility that widespread use of a Vi conjugate vaccine and high vaccination coverage in a population in which typhoid fever is endemic may lead to the emergence of otherwise rare Vi-negative variants of *S.* Typhi *(174–177)*; such variants exist and can cause typhoid fever, albeit at lower attack rates *(110, 111)*.

C.6 Safety

Current evidence suggests there are no major specific safety issues for Vi conjugate vaccines *(178)* and that reports of adverse events are similar to those of other polysaccharide–protein conjugate vaccines. It is recommended that the assessment of safety in pre-licensure studies should follow the usual approaches to ensure comprehensive monitoring and data collection *(2)*. When considering the pre-licensure safety database, the need for a sufficient sample size to estimate adverse event rates with precision is an important factor. For example, a total database of 3000 subjects across all trials and populations provides a 95% chance of observing one instance of an adverse event that occurs on average in 1 in every 1000 subjects *(179)*. Other considerations include the type of carrier protein used in the candidate Vi conjugate vaccine and the extent of clinical experience with similar conjugated vaccines.

Part D. Recommendations for NRAs

D.1 General recommendations

The guidance for NRAs and national control laboratories (NCLs) given in the WHO Guidelines for national authorities on quality assurance for biological products *(120)* and WHO Guidelines for independent lot release of vaccines by regulatory authorities *(180)* should be followed. These guidelines specify that no new biological product should be released until consistency of lot manufacturing and quality has been established and demonstrated by the manufacturer.

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety or efficacy of the Vi conjugate vaccine, should be discussed with and approved by the NRA. For control purposes, the relevant international reference preparations currently in force should be obtained for the purpose of calibrating national, regional and working standards as appropriate. The NRA may obtain from the manufacturer the product-specific or working reference to be used for lot release.

Consistency of production has been recognized as an essential component in the quality assurance of Vi conjugate vaccines. The NRA should carefully monitor production records and quality control test results for clinical lots, as well as for a series of consecutive lots of the final bulk and final product.

D.2 Official release and certification

A vaccine lot should be released only if it fulfils all national requirements and/or satisfies Part A of these WHO Recommendations *(180)*.

A summary protocol for the manufacturing and control of typhoid conjugate vaccines, based on the model summary protocol provided below in Appendix 1 and signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA/NCL in support of a request for the release of a vaccine for use. This protocol may also be referred to as the Product Specification File.

A lot release certificate signed by the appropriate NRA/NCL official should then be provided if requested by the manufacturing establishment, and should certify that the lot of vaccine meets all national requirements and/or Part A of these WHO Recommendations. The certificate should provide sufficient information on the vaccine lot, including the basis of the release decision (by summary protocol review or independent laboratory testing). The purpose of this official national lot release certificate is to facilitate the exchange of vaccines between countries and should be provided to importers of the vaccines. A model NRA/NCL Lot Release Certificate for typhoid conjugate vaccines is provided below in Appendix 2.

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Appendix 1

Model summary protocol for the manufacturing and control of typhoid conjugate vaccines

The following protocol is intended for guidance and indicates the minimum information that should be provided by the manufacturer to the NRA or NCL. Information and tests may be added or omitted as necessary with the approval of the NRA or NCL.

It is possible that a protocol for a specific product may differ in detail from the model provided. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO recommendations for a particular product should be provided in the protocol submitted.

The section concerning the final product should be accompanied by a sample of the label and a copy of the leaflet (package insert) that accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it should also be accompanied by a lot release certificate (see Appendix 2) from the NRA or NCL of the country in which the vaccine was produced and/or released stating that the product meets all national requirements as well as Part A of these WHO Recommendations.

1. Summary information on final lot

International name of product: _____
Commercial name: _____
Product licence (marketing authorization) number: _____
Country: _____
Name and address of manufacturer: _____
Nature of final product:_____
Final packaging lot number: _____
Type of container:_____
Final container lot number:_____
Number of containers in this final lot: _____
Number of doses per final container: _____
Volume of each recommended single human dose: _____
Preservative used and nominal concentration: _____

Summary of composition (*include a summary of the qualitative and quantitative composition of the vaccine per single human dose; including the conjugate, any adjuvant used and other excipients*):

_____
_____

Shelf-life approved (months):_____
Date of manufacture:_____
Expiry date:_____
Storage conditions: _____

2. Detailed information on manufacture and control

The following sections are intended for reporting the results of the tests performed during the production of the vaccine, so that the complete document will provide evidence of consistency of production. If any test had to be repeated, this information must be indicated. Any abnormal results must be recorded on a separate sheet.

Summary of source materials

It is possible that a number of bulk lots may be used to produce a single final lot. A summary of the bulk polysaccharide, activated saccharide, bulk carrier protein and bulk conjugate lots that contributed to the final lot should be provided.

Control of typhoid Vi polysaccharide

Bacterial strain

Identity of bacterial strain used
(e.g. *Salmonella* Typhi Ty2 or *Citrobacter freundii*): _____
Origin and short history: _____
Authority that approved the strain: _____
Date approved: _____

Bacterial culture media for seed-lot preparation and Vi production

Free from ingredients that form precipitate when CTAB is added: _____
Free from toxic or allergenic substances: _____
Any components of animal origin (list): _____
Certified as TSE-free: _____

Master seed lot

Lot number: _____
Date master seed lot established: _____

Working seed lot

Lot number: _____
Date working seed lot established: _____
Type of control tests used on working seed lot: _____
Date seed lot reconstituted: _____

Control of single harvests

For each single harvest, indicate the medium used; the dates of inoculation; the temperature of incubation; the dates of harvests and harvest volumes; the results of tests for bacterial growth rate, pH, purity and identity; the method and date of inactivation if used; the method of purification; and the yield of purified polysaccharide.

Control of purified typhoid Vi polysaccharide

Lot number: _____
Date of manufacture: _____
Volume: _____

Identity

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Molecular size or mass distribution

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Polysaccharide content

Date tested:_____
Method used:_____
Specification:_____
Result:_____

O-acetyl content

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Moisture content

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Protein impurity

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Nucleic acid impurity

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Phenol content

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Endotoxin content

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Residues of process-related contaminants

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Appearance

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Control of modified polysaccharide

Lot number:_____
Method of chemical modification:_____

Extent of activation for conjugation

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Molecular size or mass distribution

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Control of carrier protein

Microorganisms used

Identity of strain used to produce carrier protein:_____
Origin and short history:_____
Authority that approved the strain:_____
Date approved:_____

Bacterial culture media for seed-lot preparation and carrier-protein production

Free from ingredients that form precipitate when CTAB is added:_____
Free from toxic or allergenic substances:_____

Any components of animal origin (list):_____
Certified as TSE free:_____

Master-seed lot

Lot number:_____
Date master-seed lot established:_____

Working-seed lot

Lot number:_____
Date established:_____
Type of control tests used on working-seed lot:_____
Date seed lot reconstituted:_____

Control of carrier-protein production

List the lot numbers of harvests: indicate the medium used; the dates of inoculation; the temperature of incubation; the dates of harvests and harvest volumes; the results of tests for bacterial growth rate, pH, purity and identity; the method and date of inactivation; the method of purification; and the yield of purified carrier protein. Provide evidence that the carrier protein is nontoxic.

Purified carrier protein

Lot number:_____
Date produced:_____

Identity

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Protein impurity

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Nucleic acid impurity

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Modified carrier protein

Lot number:_____
Date produced:_____
Method of modification:_____

Extent of activation

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Control of purified bulk conjugate

Production details of bulk conjugate

List the lot numbers of the saccharide and carrier protein used to manufacture the conjugate vaccines, the production procedure used, the date of manufacture and the yield.

Tests on purified bulk conjugate

Identity

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Endotoxin content

Date tested:_____
Method used:_____
Specification:_____
Result:_____

O-acetyl content

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Residual reagents

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Vi polysaccharide content

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Conjugated and unbound (free) polysaccharide

Date tested:_____
Method used:_____

Specification:_____
Result:_____

Protein content

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Conjugation markers

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Absence of reactive functional groups (capping markers)

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Ratio of polysaccharide to protein

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Molecular size or mass distribution

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Bacterial and fungal sterility

Method used:_____
Media:_____
Volume tested:_____
Date of inoculation:_____
Date of end of test:_____
Specification:_____
Result:_____

Specific toxicity of carrier protein (where appropriate)

Method used:_____
Strain and type of animals:_____
Number of animals:_____
Route of injection:_____

Volume of injection:_____
Quantity of protein injected:_____
Date of start of test:_____
Date of end of test:_____
Specification:_____
Result:_____

pH

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Appearance

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Depending on the conjugation chemistry used to produce the vaccine, tests should also be included to demonstrate that amounts of residual reagents and reaction by-products are below a specified level.

Control of final bulk

Lot number:_____
Date prepared:_____

Preservative (if used)

Name and nature:_____
Lot number:_____
Final concentration in the final bulk:_____

Stabilizer (if used)

Name and nature:_____
Lot number:_____
Final concentration in the final bulk:_____

Adjuvant (if used)

Name and nature:_____
Lot number:_____
Final concentration in the final bulk:_____

Tests on final bulk

Bacterial and fungal sterility

Method used:_____
Media:_____

Volume tested:_____
Date of inoculation:_____
Date of end of test:_____
Specification:_____
Result:_____

Filling and containers

Lot number:_____
Date of sterile filtration:_____
Date of filling:_____
Volume of final bulk:_____
Volume per container:_____
Number of containers filled (gross):_____
Date of lyophilization (if applicable):_____
Number of containers rejected during inspection:_____
Number of containers sampled:_____
Total number of containers (net):_____
Maximum duration approved for storage:_____
Storage temperature and duration:_____

Control tests on final lot

Inspection of final containers

Date tested:_____
Method used:_____
Specification:_____
Results:_____
Appearance before reconstitution:[1] _____
Appearance after reconstitution:_____
Diluent used: _____
Lot number of diluent used: _____

Tests on final lot

Identity

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Sterility

Method used:_____
Media:_____
Number of containers tested:_____
Date of inoculation:_____

[1] This applies to lyophilized vaccines.

Date of end of test:_____
Specification:_____
Result:_____

Polysaccharide content

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Unbound (free) polysaccharide

Date tested:_____
Method used:_____
Specification:_____
Result:_____

O-acetyl content

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Molecular size or mass distribution

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Endotoxin content

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Adjuvant content and degree of adsorption (if applicable)

Date tested:_____
Nature and concentration of adjuvant per single human dose:_____
Method used:_____
Specification:_____
Result:_____

Preservative content (if applicable)

Date tested:_____
Method used:_____
Specification:_____
Result:_____

pH

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Moisture content[1]

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Osmolality

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Control of diluent (if applicable)

Name and composition of diluent:_____
Lot number:_____
Date of filling:_____
Type of diluent container:_____
Appearance:_____
Filling volume per container:_____
Maximum duration approved for storage:_____
Storage temperature and duration:_____
Other specifications:_____

Control of adjuvant[2]

Summary of production details for the adjuvant

When an adjuvant suspension is provided to reconstitute a lyophilized vaccine, a summary of the production and control processes should be provided. The information provided and the tests performed depend on the adjuvant used.

Summary information for the adjuvant

Name and address of manufacturer:_____
Nature of the adjuvant:_____
Lot number:_____
Date of manufacture:_____
Expiry date:_____

[1] This applies only to lyophilized vaccines.
[2] This section is required only when an adjuvant is provided separately to reconstitute a lyophilized vaccine.

Tests on the adjuvant

Adjuvant content

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Appearance

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Purity or impurity

Date tested:_____
Method used:_____
Specification:_____
Result:_____

pH

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Pyrogenicity[1]

Date tested:_____
Method used:_____
Specification:_____
Result:_____

Sterility

Method used:_____
Media:_____
Number of containers used:_____
Date of inoculation:_____
Date of end of test:_____
Specification:_____
Result:_____

3. Certification by the manufacturer

Name of head of production and/or quality control (typed)_____

[1] A pyrogen test of the adjuvant is not needed if a pyrogen test was performed on the adjuvanted reconstituted vaccine.

Certification by the person from the control laboratory of the manufacturing company taking overall responsibility for the production and quality control of the vaccine.

I certify that lot no. _____ of typhoid conjugate vaccine, whose number appears on the label of the final containers, meets all national requirements and satisfies Part A[1] of the WHO Recommendations to assure the quality, safety and efficacy of typhoid conjugate vaccines.[2]

Signature_____

Name (typed)_____

Date_____

4. Certification by the NRA/NCL

If the vaccine is to be exported, attach the model NRA/NCL Lot Release Certificate for typhoid conjugate vaccines (as shown in Appendix 2), a label from a final container and an instruction leaflet for users.

[1] With the exception of provisions on distribution and transport, which the NRA may not be in a position to assess.

[2] WHO Technical Report Series, No. XXXX, Annex 2.

Appendix 2

Model NRA/NCL Lot Release Certificate for typhoid conjugate vaccines

This certificate is to be provided by the NRA or NCL of the country in which the vaccine has been manufactured, on request by the manufacturer.

Certificate no._____

The following lot(s) of typhoid conjugate vaccine produced by

_____[1]

in _____,[2] whose numbers appear on the labels of the final containers, meet all national requirements[3] and Part A[4] of the WHO Recommendations to assure the quality, safety and efficacy of typhoid conjugate vaccines,[5] and comply with WHO good manufacturing practices for pharmaceutical products: main principles;[6] WHO good manufacturing practices for biological products;[7] and the WHO Guidelines for independent lot release of vaccines by regulatory authorities.[8]

The release decision is based on_____[9]

Final lot number_____

Number of human doses released in this final lot_____

Expiry date_____

[1] Name of manufacturer.
[2] Country of origin.
[3] If any national requirements have not been met, specify which one(s) and indicate why the release of the lot(s) has nevertheless been authorized by the NRA or NCL.
[4] With the exception of provisions on distribution and transport, which the NRA or NCL may not be in a position to assess.
[5] WHO Technical Report Series, No. XXXX, Annex 2.
[6] WHO Technical Report Series, No. 986, Annex 2.
[7] WHO Technical Report Series, No. 999, Annex 2.
[8] WHO Technical Report Series, No. 978, Annex 2.
[9] Evaluation of the product-specific summary protocol, independent laboratory testing and/or specific procedures laid down in a defined document, and so on as appropriate.

The certificate may also include the following information:

- name and address of manufacturer;
- site(s) of manufacturing;
- trade name and/or common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);
- type of container;
- number of doses per container;
- number of containers or lot size;
- date of start of period of validity (for example, manufacturing date);
- storage conditions;
- signature and function of the person authorized to issue the certificate;
- date of issue of certificate.

The Director of the NRA/NCL (or other appropriate authority)

Signature_____

Name (typed)_____

Date_____