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


NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Publication of this early draft is to provide information about the proposed WHO Recommendations to assure the quality, safety and efficacy of typhoid conjugate vaccines (Replacement of WHO Technical Report Series, No. 987, Annex 3) to a broad audience and to improve transparency of the consultation process.

The text in its present form does not necessarily represent an agreed formulation of the Expert Committee. Written comments proposing modifications to this text MUST be received by 6 May 2020 in the Comment Form available separately and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Department of Health Products Policy and Standards. Comments may also be submitted electronically to the Responsible Officer: Dr Richard Isbrucker at email: isbruckerr@who.int

The outcome of the deliberations of the Expert Committee will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the "WHO style guide, second edition" (KMS/WHP/13.1).

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Introduction

Guidelines on the quality, safety and efficacy of typhoid conjugate vaccines (TCVs) were developed through a series of international consultations in 2012 and 2013 and adopted by the WHO Expert Committee on Biological Standardization (ECBS) at its 64th meeting in October 2013. Since that time there have been several major developments with respect to typhoid conjugate vaccines. These include:

- 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 SAGE position paper in 2018 recommending the use of typhoid conjugate vaccines from 6 months to 45 years of age and that the introduction of TCVs in routine immunization programmes be prioritized in countries with the highest burden of typhoid disease or a high burden of antimicrobial resistant Salmonella Typhi.
- Gavi approval of funding support for the introduction of TCVs in Gavi eligible countries starting in 2019
- The WHO Prequalification in 2017 of Typbar-TCV, a typhoid conjugate vaccine from India.

The impact of these developments on the production and quality control of TCVs and on their non-clinical and clinical evaluation is reflected in the present revision. As TCVs have been licensed since the development of the original guidelines in 2013, the current document contains recommendations for their evaluation 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 guidance. 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 on-going and Part C of this document may need further updating as new data become available.

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) (1-3). 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 (4-6).
**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, and inhibits the C3 component of the complement system from fixing to the surface of *S. Typhi* (7). The Vi antigen is not unique to *S. Typhi* – it is also expressed by *S. Paratyphi* C, *Citrobacter 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 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 (8). Some of these strains are regulatory mutants that can revert to a Vi-positive state (9). However, some Vi-negative isolates from blood have been shown to harbour deletion mutations in critical genes (e.g. *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 (10).

**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 by *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 bacteremia. 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 bacteremia occurs.

Before 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 evidence 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 (i.e. excretion lasts for longer than 12 months) (11). 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 (12). They are thought to serve as a long-term epidemiological reservoir in the community, and foster transmission of typhoid wherever there is inadequate sanitation, untreated water supplies 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 (11). 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 (13). In addition, there are limited programs for detecting carriers and restricting them from handling food.

Disease burden

Variable estimates of the annual epidemiologic burden (incidence and total number of cases) of typhoid fever have been published in the scientific literature by extrapolation of data from various sources. The true incidence of typhoid fever in most regions of developing countries is not known. A study published in 2004 estimated that 22 million cases occur each year, causing 216,000 deaths, predominantly in school-age children and young adults; the annual incidence was estimated to be 10-100 per 100,000 population (14). A systematic review of population-based studies from 1984 through 2005 reported an annual incidence ranging from 13 to 976 per 100,000 persons each year based on diagnosis by blood culture (15).

More recent analysis shows 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 between 11 and 21 million typhoid fever cases and approximately 145,000 to 161,000 deaths annually (16). The majority of cases occur in Asia and Sub-Saharan Africa but many of the island nations of Oceania also experience moderate to high typhoid fever incidence and large outbreaks (17).

Several factors affect the calculation of the burden of typhoid disease, one of the most critical being how one confirms that a patient with acute febrile illness has typhoid fever. Unfortunately, there is no rapid, affordable, 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 blood culture alone identifies only 40 – 80% of the cases that are detectable using bone marrow culture (18-20). Culture of bile containing duodenal fluid and of skin snips of rose spots can be positive when blood cultures are negative (15). Prior patient treatment with antibacterial agents and the volume of blood cultured also affect the yield of cultures. Relying 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.

The incidence of typhoid, its age-specific distribution and the severity of clinical disease gleaned from passive surveillance implemented at health facilities often appears quite different from data acquired through active surveillance, during which households are visited systematically once or twice weekly to detect fever among their members and mild or early clinical illness is detected. In 2008, a study by Ochiai and colleagues reported the incidence of typhoid detected through passive surveillance (and modified passive surveillance in two countries where additional health clinics were introduced into the community) in five Asian countries (21). 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 (21). More recently, the incidence in Nepal ranged from 297 to 449 per 100,000 with greater incidences occurring during the summer months (22). Incidence data from the placebo control groups in vaccine trials also provide information on the incidence of typhoid fever in multiple geographical areas and venues. However, because vaccine efficacy trials are typically carried out in areas with high endemicity, caution must be taken when extrapolating these incidence rates to other populations. New data on age-specific occurrence in certain geographic regions, as in some sites in South Asia, confirm that typhoid fever of sufficient severity to seek medical care is common in the 1 to 4 year old age group with a large proportion of disease occurring in children between 6 months and 2 years of age (13). There has also been an increasing record of major outbreaks often associated with antimicrobial resistant S. Typhi (13). Well characterized outbreaks of confirmed typhoid fever in Sub-Saharan Africa have been reported (23–27), the increased occurrence of outbreaks due to multi drug resistant typhoidal Salmonella serovars being of particular concern. Extensively drug-resistant variants of S. Typhi have also recently emerged in India, Bangladesh and Pakistan that severely limit treatment options and are leading to a situation where the disease is increasingly difficult to treat (28, 29). The S. Typhi H58 clade, with IncHI1 plasmids carrying multidrug resistance genes and target site mutations causing fluoroquinolone resistance is responsible for much of the recent and dramatic spread of resistant strains in countries like Pakistan in 2018 (29, 30). 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 (29). The development of extensively antibiotic resistant S. Typhi, resistant to first and second line antibiotics, and its implications for disease control was reviewed in the background paper for SAGE in 2017 (16). 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, Vietnam, in 1998 strains with decreased susceptibility to fluoroquinolones increased from less than 5% to 80% within a few months (31). The large-scale outbreak of extensively drug-resistant typhoid in Pakistan demonstrates the importance of understanding the
local resistance patterns to enable the selection of appropriate antibiotics for the management of
typhoid fever cases (29).

Prior to the availability of antibacterial agents, typhoid resulted in a case-fatality rate of
approximately 10–20% (32). Current estimates covering the post antibiotic era range from 1 to
4% of those who receive adequate therapy (33). Most of the mortality occurs in developing
countries, predominantly in Asia. A review by Crump and colleagues reported community-based
mortality ranging from 0–1.8% across five studies in developing countries; hospital-based
mortality ranged from 0–13.9% (across all ages in 12 studies); and in children younger than 15
years mortality ranged from 0–14.8% (across 13 studies) (15). Hospitalization rates of 2 to 40%
have been reported indicating that the disease can be severe in a considerable proportion of
patients (21). 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 (16).

Few studies have estimated the prevalence of chronic carriers of typhoid and paratyphoid
in developing countries. A 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 (34). 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% (35). 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 (13) but thought to vary between settings of high, medium and low disease
incidence (14, 36). However, chronic carriers may represent a long-term reservoir of infection
and contribute to the persistence of typhoid fever through on-going 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%) (37). 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 2005, Huang and colleagues analysed in which systems various complications
were likely to occur: 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 haematological system (rarely) (38). Intestinal perforations leading to peritonitis and death
continue to be reported, albeit rarely, in some settings. Interestingly, the emergence of the
multiple drug 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 (2, 39).
Immune responses to natural infection

Natural typhoid infection is usually associated with the detection of serum antibodies and mucosal secretory immunoglobulin A (IgA) intestinal antibody against various *S. Typhi* antigens; cell-mediated immune responses are also measurable (40-44). In areas where typhoid is endemic, there is an age-related increase in the prevalence and geometric mean titre of anti-Vi antibodies (45). Anti-flagella (H antigen) serum IgG antibodies following natural infection are long-lived and have been studied for sero-epidemiological surveys (46).

While serological responses to LPS and flagella antigens tend to be fairly 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 (47, 48). In contrast, high concentrations of anti-Vi serum IgG antibody are detected in 80–90% of chronic carriers (47, 48).

Cell-mediated immunity also appears to play a part in protection – it has been observed that peripheral blood mononuclear leukocytes of otherwise healthy adults residing in typhoid-endemic areas, who have no history of typhoid, proliferate on exposure to *S. Typhi* antigens (49).

Disease Control

Similar to other enteric and diarrhoeal diseases, typhoid fever exists 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, as well as 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 immunization against typhoid can substantially reduce typhoid fever burden when targeted towards high risk age groups and geographic areas and when combined with improved sanitation (50). The most recent WHO guidelines for use of typhoid vaccines were published in 2018 (13).

**Typhoid vaccines:**

**Inactivated whole cell vaccine**

Inactivated *S. Typhi* bacteria (heat-inactivated and phenol-preserved) were first utilized 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 (51, 52), and documented a moderate level of efficacy lasting up to 7 years (53). Data from studies of human immune responses and immunogenicity studies in rabbits suggested that anti-H antibodies might represent an immune correlate of protection (54); later extrapolation from the results of mouse protection studies suggested that responses to Vi antigen may also correlate with protection (55, 56). However, these vaccines were associated with considerable rates of
systemic adverse reactions (57) and as they never became widely-accepted public health tools are no longer produced or marketed.

**Live, attenuated Ty21a oral vaccine**

In the early 1970s, an attenuated strain of *S. Typhi* was developed through chemical-induced mutagenesis of pathogenic *S. Typhi* strain Ty2 (55). 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 (58-62). 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 or placebo administered to about 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 (63). 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%) (58, 64). The estimate of protection was 49% (95% CI, 24–66%) with the 21-day interval between doses. Another trial used four doses administered within 7 days to Chilean schoolchildren and demonstrated even greater protection (65). 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 (66). 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 endpoints of fever and/or bacteremia as a diagnosis of typhoid (67). When efficacy was recalculated using a similar definition for typhoid diagnosis (fever with subsequent microbiological confirmation) as was used in the original vaccine/challenge study, Ty21a efficacy reached 80% (67), which is similar to that reported in the older challenge studies.

As of 2013, almost all countries where Ty21a is licensed utilize a three-dose course of enteric-coated capsules taken on alternate days, except the United States and Canada, which recommend a four-dose course.

Two other field trials in Chile (62) and Indonesia (61) compared the enteric-coated capsules with three doses of the liquid formulation. The liquid formulation conferred greater efficacy than the capsules in both trials. In Chile, where doses were given on alternate days, results with the liquid formulation were superior to Indonesia where the doses were administered 1 week apart (the point estimate of efficacy in Chile was 77%; in Indonesia it was 53%). In Chile, 78% protection was documented up to 5 years after vaccination with the liquid formulation (64). There is also indirect evidence that large-scale vaccination with Ty21a may provide some degree of protection against typhoid in people who have not been vaccinated through the mechanism of herd protection. This vaccine, first licensed in Europe in 1983 and in the USA in 1989, is approved for use in individuals older than 6 years. Because the vaccine is
highly acid labile, stomach acidity must be neutralized or by passed when Ty21a is fed orally.
The previously marketed liquid formulation is no longer produced, and only enteric coated
capsules are currently available (13).

**Vi polysaccharide vaccine**

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 (68); these vaccines are associated with low rates of febrile reactions (1–2%).
The Vi PS 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 product, Typhim Vi™, has been
prequalified by the 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 the vaccine showed a rise in anti-Vi antibody titres in adults and
children (69-71). 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 (72, 73). An epidemic of typhoid fever among French soldiers deployed in
Côte d’Ivoire showed that the risk of typhoid fever was significantly higher in persons
vaccinated more than 3 years previously (74).

Field trials in children and adults in Nepal given a single (25-µg) dose showed 72%
vaccine efficacy during 17 months of follow up (69); 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
(70). In South Africa, protection declined to 55% at 3 years (75). 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 (76). Thus, whilst a single dose of an unconjugated Vi vaccine provides moderate
protection, available data suggest that protective efficacy does not last beyond 3 years, so
revaccination is necessary within that time.

Most data suggest that children who are younger than 5 years respond poorly to Vi
polysaccharide vaccines (77). However, one cluster-randomized trial in Kolkata, India (78),
showed 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 vaccine in
Karachi, Pakistan, reported an adjusted total protective effectiveness of -38% (95% CI, -192% to
35%) for children aged 2–5 years compared with 57% (95% CI, 6% to 81%) for children aged 5–
16 years (77).

¹ https://extranet.who.int/gavi/PQ_Web/
Thus, a single dose of Vi vaccine can provide moderate protection for a limited duration, but the vaccines have the usual limitations associated with polysaccharide vaccines, including poor immunogenicity in infants and young children, short-lived immunity and lack of anamnestic immune responses to subsequent doses (72, 78, 79).

**Vi polysaccharide–protein conjugate vaccine**

Experience with several polysaccharide–protein conjugate vaccines (such as Hib, 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 experience and to try to address the limitations of the typhoid vaccines described above, several Vi polysaccharide–protein conjugate vaccines have been developed or are under development. These include Vi conjugated to tetanus or diphtheria toxoid, to CRM$_{197}$, as well as the prototype Vi conjugate vaccine with Vi conjugated to nontoxic recombinant **Pseudomonas aeruginosa** toxin (Vi–rEPA) (80). Also, a TCV that uses Vi prepared from **C. freundii** s.l. and CRM197 as the carrier protein has been shown to elicit a higher level of anti-Vi IgG compared to a licensed Vi polysaccharide vaccine control in European adults who had never been exposed to typhoid fever (81). Vi preparations from **C. freundii** s.l. have been shown to be immunologically indistinguishable from and structurally similar to those from **S. Typhi** (81, 82), although size differences have been observed for Vi polysaccharide from **S. Typhi** and **C. freundii** s.l. using size-exclusion high-performance liquid chromatography (HPLC).

Four TCVs have been licensed in India since 2008, three consisting of Vi polysaccharide conjugated to tetanus toxoid, and one to CRM$_{197}$. Other TCVs are in late-stage development in some Asian countries. Typbar-TCV (a Vi–TT vaccine) was licensed in India in 2013 for use in children over 6 months 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 (13, 83); results showed that anti-Vi antibody titres were significantly higher among recipients of TCV compared 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 followed by positive blood culture for **S. Typhi** was subsequently demonstrated in human challenge studies (84). Interim data on the efficacy of Typbar TCV in an endemic population in Nepal have also recently been published (22). These data, from a phase III participant-observer blinded randomized study in children 9 months to <16 years of age, confirm that a single dose of TCV is safe, immunogenic and effective in this field setting with an efficacy of 81.6% (95%) (22). This conclusion is supported by new data from Pakistan (VE of ~89%) and Navi Mumbai (VE of 82%) (personal communication). In view of the improved immunological properties of TCVs compared to the other available typhoid vaccines, its suitability for use in young children and expected duration of protection, the WHO SAGE recommended this as the preferred vaccine for use in all age groups and that the introduction of TCV be prioritized in countries with the highest burden of typhoid fever or with a high burden of antimicrobial resistant **S. Typhi** (13).
No internationally agreed correlates or surrogates of protection have yet been agreed for Vi conjugate vaccines, although suggested correlates have been proposed. Based on the assay used to measure anti-Vi IgG serum antibodies generated in response to the prototype US National Institutes of Health Vi-rEPA conjugate vaccine in Vietnam, a threshold value of 4.3 µg/ml anti-Vi antibody measured by ELISA was suggested to be associated with a high level of sustained protection lasting 4 years after vaccination (85, 86). A placebo-controlled, randomized, double-blind study in Vietnamese children aged 2–5 years in the highly endemic area had given an estimated efficacy of 89% for the Vi-rEPA vaccine over 46 months of follow-up (87, 88). Although the findings of Voysey and Pollard (89) confirmed that higher anti-Vi IgG levels are associated with greater protection against typhoid infection, they failed to identify a threshold level where 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 with new TCVs and current ELISA protocols to the data generated in the original Vi-rEPA trial in Vietnam (16). However, the inclusion of a working reference serum calibrated against the WHO International Standard serum anti-Vi antibodies (90, 91) can improve the interpretation of data from clinical trials. The use of the WHO International Standard enables the achievement of consistency in determining serum titers and provides a basis for the comparison of data generated by different assays and/or different laboratories.

It has been suggested that variability in 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. A recent study has identified Vi IgA as a biomarker of protective immunity against typhoid fever and quantifies the concentration of Vi IgA in vaccine recipients using the WHO International Standard (92). However, no correlate of protection was identified, and further work is 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 scale required to run field trials of efficacy and uncertainty about estimation of vaccine impact. Historically, only the chimpanzee model of the 1960s demonstrated a pathogenesis and clinical illness that somewhat recapitulated typhoid fever in humans (93-96). 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 (97-102). 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 (103, 104). The first setting during the 1950s to early 1970s involved volunteers in a penal institution (66, 105-107) whereas more recently the model has involved community volunteers (103, 104).
Part A. Guidelines on manufacture and control

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 of origin. The use of the international name should be limited to the vaccines that satisfy the specifications formulated below.

A.1.2 Descriptive definition

A typhoid conjugate vaccine 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. It should be presented as a sterile, aqueous suspension or as freeze-dried material. The preparation should satisfy all the specifications given below.

A.1.3 International reference materials

Two international standards for Vi polysaccharide (16/126 and 12/244) have been developed to measure the polysaccharide content of typhoid vaccines based on Vi polysaccharide. The Vi polysaccharide content was assessed by qNMR: standard 16/126 for Vi polysaccharide of S. Typhi has a content of 2.03 ± 0.10 mg Vi polysaccharide per ampoule, and standard 12/244 for Vi polysaccharide of C. freundii s.l. has a content of 1.94 ± 0.12 mg Vi polysaccharide per ampoule (108, 109). Both standards are suitable for physicochemical and immunoassays used for batch release of Vi polysaccharide vaccines. For example, the Vi polysaccharide standards can be used as coating antigens for in-house ELISAs (109-111). Note that a standard of homologous Vi polysaccharide should be used when analyzing the content of Vi polysaccharide vaccines (108, 109). For example, if the conjugate has been made with Citrobacter Vi polysaccharide, a Citrobacter Vi polysaccharide standard is more appropriate to use for determining its content. Use of these standards decreased the variability of in-house assays (108, 109).

International standard 16/138: serum anti-Vi polysaccharide antibodies (human), is available and consists of pooled post-immunisation sera, following vaccination with plain Vi polysaccharide or conjugated Vi polysaccharide according to the immunisation schedule of Jin et al. (84).

International standard 16/138 has been evaluated in commercial ELISAs and in-house ELISAs and has been assigned a concentration of 100 International Units (IU)/mL (110, 111). 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 (110, 111). A second collaborative study used international standards 16/138 and 12/244.
to evaluate a standardized ELISA based on a co-coating of Vi PS and poly-L-lysine and showed this generic assay to be an alternative for the commercial Vi PS ELISA (91).

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

A.1.4 Terminology

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

**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 freeze-drying (if performed). Therefore, a final lot should have been filled from a single container and freeze-dried 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 below –45 °C.

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

**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.

**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.

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2 [www.nibsc.org/products](http://www.nibsc.org/products)

3 [www.who.int/bloodproducts/catalogue](http://www.who.int/bloodproducts/catalogue)
**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 together during one production run.

**Working-seed lot:** a quantity of live strains for the production of Vi polysaccharide or the carrier protein that are of uniform composition and that have 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.

### A.2 Guidelines on general manufacturing

The general manufacturing recommendations contained in Good Manufacturing Practices: main principles for pharmaceutical products (112) and Good Manufacturing Practices for biological products (113) should be applied at establishments manufacturing Vi polysaccharide conjugate vaccines.

The production method should be shown to consistently yield Vi polysaccharide conjugate vaccines of satisfactory quality as outlined in these 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 the National Regulatory Authority’s (NRA) approval prior to implementation (114, 115).

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 (116). 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.

### A.3 Control of starting material

#### A.3.1 Certification of bacterial strain

##### A.3.1.1 Bacterial strain for preparing Vi polysaccharide

The bacterial strain used for preparing Vi polysaccharide should be from 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 (117) or a statement of risk assessment.

The strain should be capable of stably producing Vi polysaccharide. S. Typhi and C. freundii s.l. have been shown to be suitable sources for Vi polysaccharide. $^1$H nuclear magnetic resonance (NMR) spectroscopy and immunochemical tests are suitable methods for confirming the identity of the polysaccharide.

A.3.1.2 Bacterial strain for preparing the carrier protein

The bacterial strains used for preparing the carrier protein should be identified by their history, including the source from which they were obtained, number of passages and the tests used to determine the characteristics of the strains. Information regarding materials of animal origin used during passages of the bacterial strain should be provided, such as compliance with the WHO Guidelines on Transmissible Spongiform Encephalopathies in relation to Biological and Pharmaceutical Products (117) or a statement of risk assessment.

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 (see sections A.3.1.1 and A.3.1.2).

Each new seed lot prepared should be characterized using appropriate control tests to ensure comparable quality attributes to those of the previous seed lot. The control tests may include culture purity, strain identity and Vi polysaccharide structure. New seed lots should also be shown to have comparable Vi polysaccharide production in routine manufacturing prior to their use.

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, they should comply with current WHO Guidelines on Transmissible Spongiform Encephalopathies in relation to Biological and Pharmaceutical Products (117) and should be 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), 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 Requirements for Vi polysaccharide typhoid vaccine (118) and the requirements in the following sections. 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\textsubscript{2} and the final yield of Vi polysaccharide, although monitoring should not be limited to these parameters.

A.4.1.1.2 Bacterial purity

When required, 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, S. Typhi is inactivated by a suitable inactivating agent such as formaldehyde or by an alternative method such as heating. The inactivation process should be validated.

After inactivation, 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 other suitable methods approved by the NRA. All reagents should be pharmaceutical grade and sterile. Bioburden should be monitored during purification. To ensure stability, purified Vi polysaccharide in powder form should be stored at 2–8 °C, and purified Vi polysaccharide in solution should be stored below -20 °C. The duration during which the polysaccharide will remain stable should be validated.

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 by and 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 (119).

A test should be performed on the purified polysaccharide to verify its identity, such as NMR spectroscopy (120) or a suitable immunoassay, as is 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 (KD) 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 KD value or the mass distribution limits, or both, should be established and shown to be consistent from lot to lot for a given product. For gel filtration or high performance size-exclusion chromatography (HPSEC), to ensure consistency and a defined proportion of high molecular size polysaccharide, typically at least 50% of the Vi polysaccharide should elute at a KD value less than a predefined value, depending on the chromatographic method used.

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 molecular weight (MW).

Due to its high viscosity on molecular sizing columns, the Vi polysaccharide does not behave as do other polysaccharides; therefore, column matrices and eluents should be carefully chosen to ensure a representative recovery (121).

Suitable detectors for this method include a refractive index detector (122), alone or in combination with static light scattering detector (e.g. Multi-angle Laser Light Scattering, MALLS) (97) and/or with a viscometer. The methodology used should be validated to demonstrate sufficient resolution in the appropriate molecular weight range. Manufacturers are encouraged to produce Vi polysaccharide that has a consistent distribution of molecular size.

A.4.1.3.3 Polysaccharide content

The concentration of the Vi polysaccharide in its O-acetylated, acid form in eluted fractions can be measured using Hestrin’s method (123), or the acridine orange method (119, 124). More specific methods, such as NMR (120) or high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC–PAD) (124) are also acceptable, and a suitable immunoassay, for example rocket immunoelectrophoresis or ELISA, may be considered.
suitable reference preparation of Vi polysaccharide calibrated against the WHO 1st International
Standard for *Citrobacter freundii* (e.g. NIBSC 12/244) or *S. Typhi* (e.g. NIBSC 16/126) Vi
polysaccharide should be used where appropriate (see A.1.3). 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 (119, 121, 125); it should be at least 2.0 mmol/g polysaccharide (52% *O*-acyetylation), unless
justified. Hestrin’s method (123), or NMR (120, 126) or other suitable methods may be used to
quantitatively determine *O*-acylation. The methods used, and the acceptance criteria 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 agreed limits; the
methods used, and the acceptable limits 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., and using bovine serum albumin (BSA) as a reference (127). 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% of 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 cell of 1 cm path length at 260 nm is 200 (128). Other
validated methods may be used. Sufficient polysaccharide should be assayed to detect accurately
2% 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 that has been approved by the NRA. The phenol content
should be expressed in µg per mg of purified Vi antigen and shown to be consistent and within
the limits approved by the NRA.

**A.4.1.3.9 Endotoxin**
To ensure an acceptable level of pyrogenic activity in the final product, the endotoxin content of each lot of purified Vi polysaccharide should be determined and shown to be within limits agreed with the NRA. Suitable \textit{in vitro} methods include the \textit{Limulus} amoebocyte lysate (LAL) test or a monocyte activation test (MAT).

\textbf{A.4.1.3.10 Residues of process-related contaminants}

The residues of process-related contaminants in the purified polysaccharide (e.g. CTAB, formaldehyde and antifoaming agents) should be determined, and shown to be within limits agreed with the NRA. The routine testing of each lot before release for residual process-related contaminants may be omitted once consistency has been demonstrated on a number of lots; this number should be agreed with the NRA.

\textbf{A.4.1.4 Activated polysaccharide}

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

\textbf{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.

\textbf{A.4.1.4.2 Molecular size or mass distribution}

If any size-reduction or activation steps are performed, the average size or mass distribution (i.e. 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 since the size may affect the reproducibility of the conjugation process.

\textbf{A.4.2 Control of carrier-protein production}

\textbf{A.4.2.1 Consistency of microbial growth for the carrier protein}

The consistency of the growth of the microorganisms used should be demonstrated using methods such as pH and the final yield of the appropriate protein or proteins; other methods may also be used.

\textbf{A.4.2.2 Characterization and purity of the carrier protein}
Proteins that have been used as carriers in licensed conjugate vaccines include TT, DT and CRM\textsubscript{197}, but other carriers could be used. Proteins should be assayed for purity and concentration and tested to ensure they are nontoxic and appropriately immunogenic. All tests and proteins should be approved by the NRA.

For established carrier proteins, certain levels of purity may already be specified and perhaps required. Preparations of TT and DT should satisfy the relevant recommendations published by WHO (129, 130). CRM\textsubscript{197} can be obtained from cultures of \textit{Corynebacterium diptheriae} C7 /β197 (131) or expressed recombinantly by genetically modified microorganisms (132). CRM\textsubscript{197} with a purity not less than 90\% as determined by high performance liquid chromatography (HPLC) should be prepared by column chromatographic methods. The content of residual host DNA should be determined, and results should be within the limits that have been approved for the particular product by the NRA. When CRM\textsubscript{197} is produced in the same facility as DT, methods should be used to distinguish the CRM\textsubscript{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 physico-chemical assay could be performed as appropriate and convenient.

Additionally, the carrier protein should be characterized using a combination of the following physicochemical methods as appropriate: (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; (i) or mass spectrometry (133). Outcomes should be consistent with the reference material.

\textbf{A.4.2.3 Degree of activation of the carrier protein}

Adipic acid dihydrazide (ADH) or other appropriate linkers, such as N-Succinimidyl 3-(2pyridyldithio)-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 is done by using colorimetric reactions with 2,4,6-trinitrobenzenesulfonic acid and with ADH as a standard (134-136). Other suitable methods include fluorescent tagging followed by HPLC, or quadrupole time-of-flight mass spectrometry.

\textbf{A.4.3 Conjugation and purification of the conjugate}

A number of methods of conjugation are in use; all involve multistep processes (124, 134-137). Prior to demonstrating the immunogenicity of the Vi polysaccharide conjugate vaccine in clinical trials, both the methods 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 manufacturing process. The 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 methods 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 (126, 138-140). The identity of the carrier protein should also be verified using a suitable method.

A.4.4.2 Endotoxin

The endotoxin content of the purified bulk conjugate should be determined using a Limulus amoebocyte lysate (LAL) test unless otherwise justified 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 or by 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 Residual reagents

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 byproducts, 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 specifications of the process and the quantifiable methods to be used should be agreed upon in consultation with the NRA.

The process validation should also demonstrate that no significant covalent modification of the Vi polysaccharide itself has occurred, and the percentage of the modified Vi monosaccharides should not exceed what was shown to be safe and immunogenic in clinical studies. For example, many common conjugation procedures use EDC, and a frequent side reaction can result in Vi carboxylates being covalently modified to form an N-acylurea. Such modification may alter the structure of the Vi, and this modification is known to be immunogenic, leading to antibodies that cross-react with other EDC-modified polysaccharides, such as those in Hib, pneumococcal and meningococcal conjugate vaccines; thus, this modification may interfere with these vaccines. 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 (85, 108, 124), or immunological methods (e.g. rate nephelometry, rocket electrophoresis). See section A 1.3 for the recommendation for suitable reference materials.

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 RP-HPLC (SEC) (141), Capto Adhere anion-exchange resin binding (142), and deoxycholate precipitation (143), followed by HPAEC-PAD or another method as described in section A 4.4.5. Other suitable methods may be developed and validated.

A.4.4.7 Protein and unbound (free) content

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 unconjugated 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 or 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 determined 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, HP-SEC, capillary electrophoresis and other suitable methods. The conjugate should retain antigenicity for both Vi and the carrier protein as demonstrated by dot blot or western blot.

Where the chemistry of the conjugation reaction results in the creation of a unique linkage marker, such as a unique amino acid, it should be quantified for each conjugate batch to assess the extent of the covalent reaction between the Vi polysaccharide and the carrier protein. This assessment of the unique linkage marker may be omitted once the consistency of the conjugation is established with the agreement of the NRA.

A.4.4.9 Absence of reactive functional groups

The validation batch 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 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 to the size of the conjugate (121). The method should be validated and should have the specificity to distinguish the polysaccharide–protein conjugate from other components that may be present (e.g. 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 high-performance size-exclusion chromatography (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 (e.g. by using dual
monitoring of the column eluent) can provide further proof of the consistency of manufacturing
(133, 144).

A.4.4.12 Bacterial and mycotic bioburden

The purified bulk conjugate should be tested for bacterial and mycotic bioburden according to
the methods described in Part A, section 5.2, of the revised *General requirements for the sterility
of biological substances* (145), or using methods approved by the NRA. If a preservative has
been added to the product, appropriate measures should be taken to prevent it from interfering
with the test.

A.4.4.13 Specific toxicity of the carrier protein

When appropriate, the bulk conjugate should be tested to confirm the absence of specific toxicity
by the carrier protein. Alternatively, the absence of specific toxicity of the carrier protein may be
performed at the purified carrier protein stage if agreed with the NRA.

A.4.4.14 pH

If the purified bulk conjugate is a liquid preparation, the pH of each batch should be tested, and
the results should be within the range of values, determined to be safe in clinical trials, and
compatible with stability studies. For a lyophilized preparation, the pH should be measured after
reconstitution with the appropriate diluent.

A.4.4.15 Appearance

The appearance of the bulk purified conjugate should be examined. It should be clear to
moderately turbid, and colourless to pale yellow.

A.4.5 Preparation and control of the final bulk

A.4.5.1 Preparation

The final bulk is prepared by mixing a preservative or stabilizer, or both (if used), with a suitable
quantity of the bulk conjugate to meet the specifications 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 the multi-dose vaccine does not contain preservative, its use should be time-restricted
after opening of the vial. If a preservative were to be added, its effect on antigenicity and
immunogenicity must be assessed to ensure that the preservative does not affect immune
response.

A.4.5.2 Test for bacterial and mycotic sterility
Each final bulk should be tested for bacterial and mycotic sterility according to the requirements of Part A, sections 5.1 and 5.2, of the revised *General requirements for the sterility of biological substances* (145), 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 test.

### A.5 Filling and containers

The recommendations concerning filling and containers given in *Good Manufacturing Practices for biological products* (113) should be applied.

### 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, according to pharmacopoeial specifications.

#### A.6.2 Control tests on the final lot

The following tests should be performed on several final container vials or syringes from 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 the different parameters listed under this section, unless otherwise specified, should be approved by the NRA.

##### A.6.2.1 Appearance

The appearance of the final vial and final container (once reconstituted) should be verified and should meet the established criteria with respect to its form and colour.

##### 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 mycotic sterility
The contents of the final containers should be tested for bacterial and mycotic sterility according to the requirements of Part A, sections 5.1 and 5.2, of the revised General requirements for the sterility of biological substances (145), or using a method approved by the NRA. If a preservative has been added, appropriate measures should be taken to prevent it from interfering with the sterility test.

**A.6.2.3 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 is likely to be product-specific. Examples of tests that may be used include: (a) colorimetric methods; (b) chromatographic methods (including HPLC or HPAEC–PAD); or (c) immunological methods (including rate nephelometry and rocket immunoelectrophoresis).

**A.6.2.4 Unbound (free) polysaccharide**

A limit for the presence of free Vi polysaccharide should be set for each type of conjugate vaccine. Assessing the level of unconjugated polysaccharide in the final lot may be technically demanding (142); as an alternative, the molecular size of the conjugate could be determined for the final lot to confirm the integrity of the conjugate. An acceptable value should be consistent with the value seen in batches used for clinical trials that showed adequate immunogenicity; the value should be approved by the NRA.

**A.6.2.5 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 (120) or by other appropriate methods, such as Hestrin’s method (123). Routine release testing of each lot for O-acetyl content in the final product may be omitted if the NRA agrees and if the O-acetyl content is measured at the level of conjugate bulk and process validation data obtained during the product’s development confirmed that formulation and filling do not alter the integrity of the functional groups. A limit for the O-acetyl content of the Vi polysaccharide conjugate should be approved by the NRA (125).

**A.6.2.6 Molecular size or mass distribution**

The molecular size or mass distribution of the polysaccharide conjugate should be determined for each final lot using a gel matrix appropriate to 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).

**A.6.2.7 Endotoxin**
The endotoxin should be tested using a validated Limulus amoebocyte lysate test or a suitable in vitro assay. 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.

**A.6.2.8 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 amount of aluminium 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 used, and the specifications should be approved by the NRA.

**A.6.2.9 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.6.2.10 General safety (innocuity)**

The general safety test (also referred to as the abnormal toxicity test or innocuity test) is no longer recommended. The Expert Committee on Biological Standardization (ECBS) has recommended the discontinuation of the innocuity test listed in all WHO Recommendations, Guidelines and manuals for biological products (146).

**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 found for lots shown to be safe and effective in clinical trials. For a lyophilized preparation, the pH should also be measured after reconstitution with the appropriate diluent.

**A.6.2.12 Moisture content**
If the conjugate is dried, the acceptable level of residual moisture should be established, and the limit should be agreed with the NRA.

### A.6.2.13 Osmolality

The osmolality of the final lots should be determined and shown to be within the limits agreed with the NRA.

### A.6.3 Control of diluents

The recommendations in *Good Manufacturing Practices: main principles for pharmaceutical products* (112) should apply to the manufacture and quality control of the diluents used to reconstitute conjugate typhoid vaccines. 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 the appearance, identity, volume, sterility and content of key components.

### A.7 Records

The recommendations in *Good Manufacturing Practices for biological products* (113) should be followed as appropriate for the level of development of the candidate vaccine.

### A.8 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 recommendations in *Good Manufacturing Practices for biological products* (113) that are appropriate for a candidate vaccine should be applied, and the following additional information should also be included.

The label on the cartons enclosing one or more final containers, or the leaflet accompanying each container, should include:

- a statement that the candidate vaccine fulfils Part A of these Guidelines;
- the information that if the vaccine is a lyophilized form it should be used immediately after reconstitution; if data have been provided to the licensing authority to indicate 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 manufacturer.
A.10 Distribution and shipping

The recommendations appropriate for candidate vaccines given in Good Manufacturing Practices for biological products (113) should be followed. Shipments should be maintained within specified temperature ranges, and packages should contain cold-chain monitors (147).

A.11 Stability, storage and expiry date

The relevant recommendations outlined in Good Manufacturing Practices for biological products (113) should be followed. The 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 WHO Guidelines on stability evaluation of vaccines (148) and WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (149). 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 in section below. Different specifications should be established for release and end of shelf-life as described in WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (149). The specification at the end of shelf-life should be linked to lots demonstrated to be safe and effective/immunogenic in clinical trials, while the release specification is often based on manufacturing capability.

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 conditions of storage. The hydrolysis may result in a 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, or in a reduced molecular size of the conjugate, or some combination of these.

The O-acetyl content should be monitored quantitatively for stability testing and release testing. The quantity of free protein should be monitored for stability testing and release testing. The molecular size or mass distribution should be monitored for stability testing and release testing.

If applicable, the residual moisture should be monitored for 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 as 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.

If manufacturers consider incorporating a vaccine vial monitor (VVM) into the label, they should provide appropriate data to justify a correlation between the stability kinetics of the vaccine and the selected VVM (150).

A.11.2 Storage conditions

The recommended long-term storage conditions should be based on the findings of the stability studies and ensure that all stability-indicating parameters (e.g. free saccharide) of the conjugate vaccine meet the 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, use of a vaccine under ECTC requires specific monitoring as described in Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (149).

A.11.3 Expiry date

The expiry date should be based on the shelf-life supported by stability studies and approved by the NRA. The start of the dating period, e.g. the date of formulation of final bulk or the date of filling, should be agreed with the NRA. The expiry dates for vaccines and diluents may be different from one another.

A.11.4 Expiry of reconstituted vaccine (if applicable)
For single-dose containers, the reconstituted vaccine should be used immediately. For multidose containers, the use of reconstituted container should follow WHO Multi-dose vial policy (Internet at: http://www.who.int/immunization/documents/en/), and shall be according to the Summary of Product Characteristics and supplied instructions.
Part B. Nonclinical evaluation of new 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 (151), and they should be read in connection with Part B of these Recommendations. Specific issues to be considered in relation to candidate Vi conjugate vaccines are considered in section B.3. Plans for nonclinical studies to be conducted during the development of the vaccine should be discussed with the NRA early in 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 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 development. Vaccine lots used in nonclinical studies should be adequately representative of those intended for use in clinical investigations – that is, the safety data should support the initiation of clinical studies in humans. Ideally, the lots 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

Immunogenicity studies in animal models may be poorly predictive of the efficacy of glycoconjugate vaccines in humans and should only be conducted when they provide proof-of-concept information in support of a clinical development plan. Immunogenicity data derived from appropriate 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 that will be evaluated in clinical trials. However, any animal test plan should incorporate 3Rs (Replace, Reduce, Refine) best practices. When animal models are used for 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, non-conjugated Vi vaccine), and a booster response should occur after the second dose (134). Immunogenicity studies of Vi polysaccharide conjugates have been conducted in mice (97, 124, 152-154); in humans, correlation has been made between the level of anti-Vi IgG and protection against clinical disease (69, 86, 155). Therefore, the primary end-point for nonclinical studies of the immunogenicity of Vi conjugate vaccines should be the level of anti-Vi elicited.
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 the functional immune response (e.g. by evaluating serum bactericidal antibodies, opsonophagocytic activity and serum-dependent opsonophagocytic killing) (see section C.2.2). These studies may also address the interference that can occur among antigens when multiantigen vaccines are used (see section C.2.3). In such cases, the response to each antigen should be evaluated.

Although there have been advances in 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 that the production batches have the same protective efficacy as those used and shown to be protective in clinical trials. Therefore, emphasis is increasingly placed on ensuring consistency in manufacture through the use of modern physical, chemical and immunological quality-control methods. Once validated, these non-animal methods are considered more appropriate for use in lot release processes (Part A).

**B.4 Nonclinical toxicity and safety**

WHO Guidelines on nonclinical evaluation of vaccines (151) should be followed when assessing toxicity and safety. Toxicity studies for Vi polysaccharide conjugate typhoid vaccines may be performed 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. These effects should be assessed for a wide range of doses, including those exceeding the intended clinically relevant dose. If novel proteins are used to manufacture conjugate vaccines, toxicity studies should be performed on these proteins first. Nonclinical safety studies should be conducted in accordance with the GLPs that have been described elsewhere (156, 157). For ethical reasons, it is desirable to apply the 3Rs concept of “Replace Reduce Refine” to minimize the use of animals in research where scientifically appropriate.
Part C. Clinical evaluation of new typhoid conjugate vaccines

C.1 General considerations for clinical studies

The general principles described in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (158) apply to Vi polysaccharide conjugate vaccines and should be followed. Some issues specific to the clinical development program for Vi conjugate vaccines are discussed below and should be read in conjunction with the general guidance mentioned above. WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (159) are also available and should be consulted.

Vi conjugate vaccines have already been licensed in some countries for use in children over 6 months and adults up to 45 years of age and one has been prequalified by WHO (13). The licensure of effective Vi conjugate vaccines in some countries and their availability through the WHO prequalification program has 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 (22, 83, 160-163). The principles for clinical evaluation outlined below are based on the current situation and should be read in light of the circumstances in any one NRA’s jurisdiction.

C.2 Outline of the clinical development program

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

C.2.1 Dose and Schedule

The early clinical development program 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, since experience with other conjugated polysaccharide vaccines has indicated that differences in conjugation chemistry can affect immune responses to the polysaccharide(s).
In the absence of a pre-licensure efficacy study, pathways to approval of a candidate Vi conjugate vaccine in any one NRA’s jurisdiction may depend on the following:

- If there is a licensed Vi conjugate vaccine for which protective efficacy has been documented (these data may come from pre- and/or post-licensure efficacy studies and/or from post-approval 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, approval of a candidate Vi conjugate vaccine may be based on immunogenicity studies in which vaccine efficacy is inferred by estimating the proportion of vaccinated subjects predicted to be protected on the basis of immune responses that exceed the concentration identified. In this situation, it may still be appropriate for a 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 efficacy of a candidate conjugate vaccine by comparing the immune response with a licensed unconjugated Vi vaccine in subjects aged from 2 years. See further in section C4.

C.3 Assessment of the immune response

C.3.1 Immune parameters of interest

The primary parameter for assessing the humoral immune response to a vaccine is usually based on a measure of functional antibody. However, 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.

The assessment of the immune response to licensed unconjugated (78, 164, 165) and conjugated (22, 84, 85, 166) Vi vaccines has predominantly used ELISA methods to measure total anti-Vi IgG in serum. For unconjugated Vi polysaccharide vaccines, approval has often been based on comparing proportions that achieve anti-Vi IgG at least 1 µg/mL and/or on the proportions that achieve at least a 4-fold increase in anti-Vi IgG from pre- to post-vaccination (22). A regional or in-house working reference serum preparation calibrated against the WHO International Standard serum anti-Vi antibodies (see Part A.1.6) should be used in the interpretation of immunogenicity data from clinical trials. The use of the WHO International
Standard improves consistency in the determination of serum titers 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, although correlations between total serum antibody (75), total anti-Vi IgG (77, 87, 88, 167, 168), or anti-Vi IgA (92) 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 but 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\textsubscript{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\textsubscript{197}, DT or TT as the carrier protein, there is some potential 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 (158).

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 has elapsed. Details of the clinical assessment of priming may be found in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (158). Whether or not booster doses will be needed to maintain protection after successful priming with Vi conjugate vaccines is not yet known. Until such time as this question is answered, there should be plans 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 (158). 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 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 to the candidate vaccine when compared with a licensed Vi conjugate vaccine. The primary immune parameter for 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 pre-defined and agreed between NRAs and sponsors.

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

If the sponsor wishes to, or is requested to conduct a comparative study against a licensed Vi conjugate for which efficacy is not documented, demonstrating non-inferiority for the candidate vs. licensed vaccine does not inform on the potential efficacy of the candidate vaccine. Therefore, either the immune responses to the candidate 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 conjugated Vi vaccines with unconjugated Vi vaccines

Studies that compare candidate Vi conjugate vaccines with licensed unconjugated Vi vaccines should only be conducted in subjects who are aged at least 2 years. It is recommended that such studies are conducted only if a licensed Vi conjugate vaccine comparator is not available, but it is perceived important to generate comparative safety and immunogenicity data vs. a licensed vaccine (see section C.2). 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.

See section C.3. regarding possible approaches to the primary comparison of immune responses.

The immune responses should be measured in samples collected at day 28 after the initial vaccination series have been completed (i.e. 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 vaccinated groups with unvaccinated groups
There are two situations in which such studies may be considered:

i) As explained in section C.2, 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 some context.

ii) 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 who are younger than 2 years. A comparison between a candidate Vi conjugate vaccine and a licensed Vi polysaccharide vaccine would not be appropriate due to lack of reliable protective immune responses to the latter in children aged < 2 years. In this setting, it is recommended that studies employ randomized allocation to the candidate Vi conjugate vaccine (i.e. the vaccinated group) or to a licensed non-typhoid vaccine from which study subjects may derive some benefit (i.e. the control group).

The anti-Vi immune responses in the group receiving the candidate Vi conjugate vaccine should be superior to those in the control (unvaccinated) group. To put the immune responses observed into context, anti-Vi titres may be compared with one or both of:

- the immune response to an unconjugated Vi vaccine in subjects > 2 years
- the immune response to the candidate Vi conjugate vaccine in subjects aged > 2 years

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 (158).

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 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. A double-blind design is recommended but this would require that the control group is randomized to a licensed Vi conjugate vaccine, if available, or unconjugated Vi vaccine if appropriate for the age group selected. Otherwise, a non-typhoid vaccine that is indistinguishable in appearance from the candidate conjugate vaccine and is administered in the same way (i.e. route and schedule) may be considered.

Successful typhoid challenge studies conducted in healthy adults using an appropriate and validated model (i.e. one in which some protective efficacy of unconjugated Vi 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 (104). Healthy adults that are expected or known to be naïve 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 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 immunization coverage in a population where typhoid fever is endemic may lead to the emergence of otherwise rare Vi-negative variants of S. Typhi (169-172); these variants exist and can cause typhoid fever, albeit they have lower attack rates (105, 106).

C.6 Safety

Current evidence suggests there are no major specific safety issues for Vi conjugate vaccines (173) and that reports of adverse events are similar to those of other polysaccharide 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 (158).
Part D. Guidelines for NRAs

D.1 General guidelines

The general guidelines for NRAs and national control laboratories (NCLs) given in the WHO Guidelines for national authorities on quality assurance for biological products (114) and the WHO Guidelines for independent lot release of vaccines by regulatory authorities (174) should be followed. These Guidelines specify that no vaccine substance or product should be released until the manufacturer has demonstrated consistency in manufacturing and quality.

The detailed procedures for production and quality control, and any significant changes in these that may affect the quality, safety or efficacy of a Vi polysaccharide conjugate typhoid 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 the product-specific or working reference from the manufacturer for use in lot release testing.

Consistency of production has been recognized as an essential component in ensuring the quality of vaccines. The NRA should carefully monitor production records and the results of quality-control tests for clinical lots, as well as results 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 the national requirements and Part A of these Recommendations (174).

A model protocol for the manufacturing and control of TCVs is shown in Appendix 1; this protocol should be signed by the responsible official of the manufacturing establishment and should be prepared and submitted to the NRA in support of a request to release the vaccine for use. This protocol may also be referred to as the Product Specification File.

A Lot Release Certificate signed by the appropriate official of the NRA should be provided to the manufacturing establishment and should certify that the lot of vaccine meets all national requirements and/or Part A of these Recommendations. The certificate should also state the lot number, the number under which the lot was released, and the number appearing on the labels of the containers as well as the basis of the release decision (by summary protocol review or independent laboratory testing). In addition, the date of the last satisfactory determination of critical quality parameters (such as the ratio of free Vi polysaccharide to bound Vi polysaccharide) as well as the expiry date assigned on the basis of the shelf-life of the vaccine should be stated. A model NRA Lot-release Certificate is given in Appendix 2. A copy of the model protocol should be attached to the lot-release certificate – the purpose of which is to facilitate the exchange of TCVs between countries.
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Appendix 1

Model 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 an NRA. Information and tests may be added or omitted as required by an NRA, and should include information if the intermediates, drug substances, drug product, or final vaccine is manufactured by more than one commercial entity.

It is thus 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 given in the protocol submitted.

The section concerning the final product must be accompanied by a sample of the label and a copy of the leaflet that accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it must also be accompanied by a lot-release certificate from the NRA of the country where the vaccine was produced, stating that the product meets national requirements as well as the recommendations in Part A of this document.

Summary information on the final lots:

International name of product: ____________________________________________
Commercial name: ______________________________________________________
Product license (marketing authorization) number: ____________________________
Country: ____________________________
Name and address of manufacturer: ________________________________________
Final packing lot number: ________________________________________________
Type of containers: _______________________________________________________
Number of containers in this packing lot: _________________________________
Final container lot number: _____________________________________________
Number of filled containers in the final lot: _________________________________
Date of manufacture: ____________________________________________________
Nature of final product: __________________________________________________
Preservative used and nominal concentration: ______________________________
Volume of each recommended single human dose: _________________________
Number of doses per final container: ______________________________________
Summary of composition: ________________________________________________

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

Shelf-life approved (months): ____________________________________________
Expiry date: ____________________________
Storage conditions: ____________________________________________________

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 in
production; thus, if any test had to be repeated, this information must be indicated. Any
abnormal results should be recorded on a separate sheet.

**Detailed information on manufacture and control**

**SUMMARY OF STARTING 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 *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; 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
<table>
<thead>
<tr>
<th>Date tested:</th>
<th>Method used:</th>
<th>Specification:</th>
<th>Result:</th>
</tr>
</thead>
<tbody>
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</table>

### Phenol content

<table>
<thead>
<tr>
<th>Date tested:</th>
<th>Method used:</th>
<th>Specification:</th>
<th>Result:</th>
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<tbody>
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</table>

### Endotoxin content

<table>
<thead>
<tr>
<th>Date tested:</th>
<th>Method used:</th>
<th>Specification:</th>
<th>Result:</th>
</tr>
</thead>
<tbody>
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</table>

### Residues of process-related contaminants

<table>
<thead>
<tr>
<th>Date tested:</th>
<th>Method used:</th>
<th>Specification:</th>
<th>Result:</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

### Control of modified polysaccharide

<table>
<thead>
<tr>
<th>Lot number:</th>
<th>Method of chemical modification:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tbody>
</table>

### Extent of activation for conjugation

<table>
<thead>
<tr>
<th>Date tested:</th>
<th>Method used:</th>
<th>Specification:</th>
<th>Result:</th>
</tr>
</thead>
<tbody>
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</table>

### Molecular size or mass distribution

<table>
<thead>
<tr>
<th>Date tested:</th>
<th>Method used:</th>
<th>Specification:</th>
<th>Result:</th>
</tr>
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<tbody>
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</tbody>
</table>

### CONTROL OF CARRIER PROTEIN

**Microorganisms used**

<table>
<thead>
<tr>
<th>Identity of strain used to produce carrier protein:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>
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: ________________________________
<table>
<thead>
<tr>
<th><strong>Result</strong></th>
<th></th>
</tr>
</thead>
</table>

**Residual reagents**

<table>
<thead>
<tr>
<th>Date tested:</th>
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<tbody>
<tr>
<td>Method used:</td>
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<tr>
<td>Specification:</td>
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<tr>
<td>Result:</td>
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</tbody>
</table>

**Vi polysaccharide content**

<table>
<thead>
<tr>
<th>Date tested:</th>
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<tbody>
<tr>
<td>Method used:</td>
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<td>Specification:</td>
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<tr>
<td>Result:</td>
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</tbody>
</table>

**Conjugated and unbound (free) polysaccharide**

<table>
<thead>
<tr>
<th>Date tested:</th>
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</thead>
<tbody>
<tr>
<td>Method used:</td>
<td></td>
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<tr>
<td>Specification:</td>
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<tr>
<td>Result:</td>
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</tbody>
</table>

**Protein content**

<table>
<thead>
<tr>
<th>Date tested:</th>
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<tbody>
<tr>
<td>Method used:</td>
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<tr>
<td>Specification:</td>
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<td>Result:</td>
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</table>

**Conjugation markers**

<table>
<thead>
<tr>
<th>Date tested:</th>
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<tbody>
<tr>
<td>Method used:</td>
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<td>Specification:</td>
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<td>Result:</td>
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</table>

**Absence of reactive functional groups (capping markers)**

<table>
<thead>
<tr>
<th>Date tested:</th>
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<tbody>
<tr>
<td>Method used:</td>
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<tr>
<td>Specification:</td>
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<tr>
<td>Result:</td>
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</tbody>
</table>

**Ratio of polysaccharide to protein**

<table>
<thead>
<tr>
<th>Date tested:</th>
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<tbody>
<tr>
<td>Method used:</td>
<td></td>
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<tr>
<td>Specification:</td>
<td></td>
</tr>
<tr>
<td>Result:</td>
<td></td>
</tr>
</tbody>
</table>
**Molecular size or mass distribution**

- Date tested: 
- Method used: 
- Specification: 
- Result: 

**Bacterial and mycotic bioburden**

- 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: 
- 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 mycotic 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:**
- **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:**
- **Date of end of test:**
- **Specification:**
- **Result:**

### Polysaccharide content

- **Date tested:**
- **Method used:**
- **Specification:**
- **Result:**

### Unbound (free) polysaccharide

- **Date tested:**
- **Method used:**
- **Specification:**
- **Result:**

---

4 This applies to lyophilized vaccines.
O-acetyl content

Date tested: ________________________________

Method used: ________________________________

Specification: ________________________________

Result: ________________________________

Molecular size or mass distribution

Date tested: ________________________________

Method used: ________________________________

Specification: ________________________________

Result: ________________________________

Endotoxin or pyrogen 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: ________________________________

General safety

Date tested: ________________________________

Method used: ________________________________

Specification: ________________________________

Result: ________________________________

pH

Date tested: ________________________________

Method used: ________________________________

Specification: ________________________________
Result: __________________________________________

Moisture content
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

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: _______________________________________

Tests on the adjuvant

---

5 This applies only to lyophilized vaccines
6 This section is required only when an adjuvant is provided separately to reconstitute a lyophilized vaccine.
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

Date tested: 
Method used: 
Specification: 
Result: 

Sterility

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

CERTIFICATION BY THE MANUFACTURER

7 A pyrogen test of the adjuvant is not needed if a pyrogen test was performed on the adjuvanted reconstituted vaccine.
Name of head of quality control (typed) ____________________________________________________________________

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/or satisfies Part A of WHO Recommendations on the quality, safety and efficacy of typhoid conjugate vaccines (20).  

Signature ____________________________________________________________________

Name (typed) ____________________________________________________________________

Date ____________________________________________________________________

Certification by the NRA

If the vaccine is to be exported, attach a Lot-release Certificate from the NRA (as shown in Appendix 2), a label from a final container and an instruction leaflet for users.

---

8 With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.

Appendix 2

Model NRA Lot-release Certificate for typhoid conjugate vaccines

Certificate no. ____________________________

The following lot(s) of typhoid conjugate vaccine produced by ____________________________ in
__________________ ____________________________
whose numbers appear on the labels of the final containers, meet all national requirements
and Part A of the WHO Recommendations on the quality, safety and efficacy of typhoid conjugate vaccines (2020)
and complies with WHO Good manufacturing practices: main principles for pharmaceutical products;
Good manufacturing practices for biological products; and Guidelines for independent lot release of
vaccines by regulatory authorities.

The release decision is based on ________________________________________________________________

Final lot number: ____________________________
Number of human doses released in this final lot: ____________________________
Expire date: ____________________________

The Director of the NRA (or other appropriate authority)

Name: (typed) ____________________________
Signature: ____________________________
Date: ____________________________

10 Name of manufacturer
11 Country of origin.
12 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.
13 With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.
18 Evaluation of the summary protocol, independent laboratory testing, or procedures specified in a defined document etc., as appropriate