I. About the Disease and Pathogen

Basic information on pathogen, including transmission, estimated global disease burden for those at risk, for morbidity and for mortality, including uncertainties/data gaps, geographical distribution, economic burden if available, age groups affected and target groups for vaccination.

Existing preventive, diagnostic and treatment measures and their limitations.

The genus *Salmonella* belongs to the family of Enterobacteriaceae and are Gram-negative, non-spore-forming, facultative anaerobic bacilli (1). *Salmonella enterica* serovar Typhi (*S. Typhi*) and *Salmonella enterica* serovar Paratyphi (*S. Paratyphi*) A and B cause enteric fever, a systemic febrile illness, occurring only in humans that is distinguished from the more commonly self-limited acute gastroenteritis caused by the other numerous *Salmonella* serotypes. The remaining serovar or serotypes of the *Salmonella* genus comprise the group of nontyphoidal *Salmonella* (NTS), which infect a variety of hosts and are frequently zoonotic (2). Of the more than 2,500 NTS serovars, *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S. Typhimurium*) and *Salmonella enterica* serovar Enteritidis (*S. Enteritidis*) account for nearly 80 percent of all human isolates of NTS reported globally (3). NTS has been increasingly recognized recently as a major cause of invasive bacterial infections in young children and HIV-infected individuals in sub-Saharan Africa, as well as elderly and immunocompromised individuals worldwide (3).

The global incidence of NTS gastroenteritis in 2010 was estimated to be 93 million cases, some 80.3 million of which were via food-borne transmission, with 155,000 deaths. The economic burden of NTS is significant in the developed world. In the United States alone, NTS costs US$3.3 billion per year, with a loss of 17,000 quality-adjusted life years, the most of any food-borne pathogen (3). As mentioned previously, NTS can also cause severe extra-intestinal, invasive bacteremia, which is referred to as invasive nontyphoidal salmonellosis (iNTS) (2). Invasive infections of *Salmonella* are more common throughout the developing world and have become the most common cause of bacteremia in tropical Africa, especially among young children and individuals with HIV (4). It usually presents as a febrile illness. In fact, iNTS frequently occurs without gastrointestinal symptoms in both adults and children. Symptoms of iNTS are similar to malaria and include fever and sweats (more than 90 percent) as well as splenomegaly (40 percent). It is not clear why iNTS is such a problem in Africa, but this could be related to: increased invasiveness of the distinct clades of iNTS bacteria (such as *S. Typhimurium* ST313) that are found in Africa and not elsewhere; decreased host immunity related to HIV infection, malaria, and malnutrition; and increased opportunities for human-to-human transmission, e.g., through contaminated water supplies, and NTS bacteremia in HIV-infected African adults has an associated high mortality (up to 47 percent) and recurrence rate (43 percent) rate (5).

Because of the non-specific symptoms associated with iNTS infection, clinical guidelines and diagnosis remain difficult, especially in resource-poor settings. Blood or bone-marrow culture can be used for diagnosing cases of bacteremia. Enzyme-linked immunosorbent assay (ELISA) of blood serum is available for detecting antibodies to subspecies of *Salmonella enterica* serovars, but due to the ubiquitous presence of such antibodies, ELISA cannot be used to diagnose iNTS infections (6). Polymerase chain reaction (PCR) has been used for rapid diagnosis of *Salmonella* from stool samples, and a multiplex PCR has been proposed as a means to identify invasive *Salmonella* disease in West Africa. However, these techniques have not been validated in routine clinical practice. Quantitative real-time PCR is another methodology that is used to both amplify and quantify the amount of a specific strand of bacteria-specific DNA in a sample. A recently developed method, “MAMEF” (microwave-accelerated metal-enhanced fluorescence), could potentially be developed for the rapid diagnosis (less than 30 seconds) of iNTS at very low infective loads (1 CFU/mL) (7).
Antibiotics are used to treat iNTS, and the choice of antimicrobials and length of treatment are determined by the cost and availability of antibiotics, local pattern of resistance, and a patient’s treatment response. Treatment of iNTS has become more difficult as antimicrobial resistance has been increasingly identified. Treatment of iNTS in HIV-infected individuals is further complicated, due to the high rates of recurrence. It is important to treat such individuals using antibiotics with optimal intracellular penetration, such as fluoroquinolones (8), but resistance to fluoroquinolones and ceftriazone is increasing.

The burden of iNTS is likely to increase around the world. Moreover, as available vaccines increasingly control Streptococcus pneumoniae and Haemophilus influenzae, NTS will be responsible for an increasingly larger proportion of cases of bacteremia. As mentioned above, the increased burden of disease from iNTS in Africa has been associated with the appearance of a new clade of S. Typhimurium, known as ST313, which has acquired multidrug resistance. As available tools for treatment become less effective, the problem of iNTS is likely to continue to increase, making vaccine development an important priority for disease control efforts (2).

II. Overview of Current Efforts

A. Biological feasibility for vaccine development
Evidence that vaccine development is biologically feasible including from development of naturally acquired immunity, from vaccine development for related pathogens, from animal models or in vitro data

In addition to the fact that vaccines against one particular serotype of Salmonella (S. Typhi) are available, specific evidence from animal and human studies support the feasibility of vaccine development against NTS and iNTS. It is known that both antibodies and complement can kill Salmonella species in vitro. Epidemiological studies in sub-Saharan Africa have shown that acquisition of antibodies against iNTS corresponds with a decrease in age-related incidence of disease, and that serum antibodies have corresponding in vitro functional activity in killing bacteria and mediate intracellular oxidative killing of iNTS (9, 10). This supports the potential that vaccines against key NTS serovars could be designed to induce protective antibodies (11). One study from Africa found that very high titers of antibodies directed against Salmonella lipopolysaccharide (LPS) were associated with impaired in vitro serum killing of S. Typhimurium in a proportion of HIV-infected Malawian adults (12). The in vivo significance of these findings is not clear, since anti-LPS antibodies have bactericidal activity and protect against NTS challenge in mouse models. Animal proof-of-concept has been demonstrated with live attenuated vaccines, experimental O-antigen based conjugate vaccines, as well as preclinical formulations based on purified proteins of flagellin and various iNTS outer membrane proteins.

B. General approaches to vaccine development for low- and middle-income markets
What are the scientific approaches and indications and target/age/geographic groups being pursued? What public health needs will these vaccines meet if successfully developed? Where there are several different possible indications/target groups, how much consensus is there as to prioritization between these for vaccine development in LMIC.

Vaccine strategies target the cell wall O-antigens, as well as surface and outer membrane proteins of S. Typhimurium and S. Enteritidis. Highly favored is the subunit glycoconjugation approach, despite the fact that unlike S. Typhi, the NTS are not encapsulated. More recently, a novel delivery strategy employing the Generalized Modules for Membrane Antigens (GMMA) bacterial particle technology to deliver surface polysaccharide and outer membrane proteins in correct conformation and orientation is also being developed. This technology has self-adjuvanting activity through the co-delivery of multiple pathogen-associated molecular patterns (PAMP). If successful, with its reported potential for high yield and simplified production distinguishing it from the complex conjugation process, could be particularly
relevant in the developing country context.

An alternative subunit approach is the development of recombinant or purified protein vaccines based on surface or outer membrane protein antigens, such as flagellin and porins OmpC, F and D. The advantage of this approach is the ability to target more conserved protein antigens and the potential for achieving broad-spectrum coverage by selecting a combination of subunit antigens from clinically important serovars, something that a reverse vaccinology approach using bioinformatics analysis of whole genome sequences may enable. However lack of precise knowledge regarding the critical B cell and T cell epitopes of Salmonella as well as manufacturing issues related to producing proteins with appropriate conformation or challenges related to protein purification complicate development.

Finally other vaccine approaches against iNTS include various live attenuated candidates which could be promising, given the ability of these vaccines to induce excellent mucosal immunity and the ease of oral delivery. In addition, molecular techniques and genome sequencing allow now for the ability to rationally tailor attenuation with genetic modifications and mutations. The challenge remains the careful balancing between immunogenicity and reactogenicity, and the importance of assuring reliable and adequate attenuation especially if development is targeting populations with a high prevalence of immune-compromised conditions such as HIV in Africa.

Vaccines for iNTS will need to target infants between the ages of two and four months old, before peak incidence occurs around the age of 12 months. In addition, vaccine implementation would likely also include populations infected with HIV, as they are at heightened risk of infection with NTS. In developed countries, NTS vaccines should also target the elderly who experience very high case-fatality rates (up to 50 percent). It has been proposed that, in children, programmatic field implementation would integrate directly with existing Expanded Programme on Immunization schedules, perhaps at 6, 10, and 14 weeks.

III. Technical and Regulatory Assessment

Highlight perceived positive/negative aspects in clinical/regulatory pathways e.g. well established product development and regulatory pathway to licensure, accepted immune correlates and/or functional assays, accepted surrogate efficacy endpoints, existence of well accepted animal or challenge models, agreed trial designs and endpoints. Possibilities to develop case for correlates/surrogates should be included.

In 2013, the World Health Organization produced guidance on the regulation and prequalification of typhoid conjugate vaccines (WHO/BS/2013.2215). Although no such pathway is available for NTS vaccines, this framework will likely provide a strong model for how to efficiently develop such guidelines once they are available.

Murine models are available for iNTS, as S. Typhimurium causes a systemic infection in susceptible mice. In the mouse model, S. Typhimurium enters through the gut mucosa and spreads via the lymphatic system. In untreated mice, infection manifests as invasive disease without gastroenteritis. To produce an enterocolitis infection in mice, they must be pre-treated with streptomycin or other antibiotics prior to infection with NTS. There are also other important differences between mice and humans, such as the inability of mouse complement to kill NTS in vitro. Although there is not yet a published correlate of protection, there are in vitro assays that are able to quantify the serum bactericidal antibody (SBA) activity induced by S. Typhimurium and S. Enteritidis, such that vaccine development against these bacteria can be facilitated. Assays have also been developed to assess the opsonophagocytic and killing activity of anti-Salmonella antibodies (9).

IV. Status of Vaccine R&D Activities

Summarize status of vaccine design, pre-clinical and clinical trial activity, including platforms, vectors, and adjuvants. Note academic, government, biotech and industry entities engaged. Summarize antigenic targets (if
subunit approaches). Section on major advances in last 3-5 years, including key opportunities highlighted by recent science developments in the area.

Several vaccines for the most common strains of NTS, namely S. Typhimurium and S. Enteritidis, are under development. Bivalent vaccines for both strains are being developed under several different approaches. As of yet, it is unclear if these vaccine candidates will be protective against both the gastroenteritis and invasive manifestations of iNTS, though the ultimate goal of these vaccines is to reduce childhood mortality in the developing world. It has been proposed that a multivalent vaccine composed of five to six conjugates could protect against the most relevant forms of invasive Salmonella enterica (9).

The Center for Vaccine Development at the University of Maryland, Baltimore (UMB) is developing a live attenuated, oral bivalent NTS vaccine candidate. They have developed attenuated strains of both S. Typhimurium (CVD 1931, derived from a wild-type S. Typhimurium strain from the ST313 genotype circulating in sub-Saharan Africa) and S. Enteritidis (CVD 1944, derived from wild-type invasive S. Enteritidis). These live strains elicit significant seroconversion (four-fold or greater rise) of functional anti-LPS and anti-flagellin antibody titers. Furthermore, CVD 1921, a prototype strain, has been confirmed to be adequately safe and well tolerated in immunocompromised model animals (chronic simian immunodeficiency virus-infected rhesus macaques) (13).

A bivalent, NTS conjugate vaccine candidate has also been developed by UMB. This vaccine is based on covalently linking the core and O-polysaccharides (COPS) of S. Typhimurium and S. Enteritidis, respectively, to the Phase 1 flagellin subunits of those serovars. It has been shown that flagellin elicits a good antibody response, and flagellin alone is protective. UMB has created reagent strains of S. Typhimurium and S. Enteritidis that hyper-express flagellin. These reagent strains are valuable for the economical and safe purification of components for the NTS COPS-FliC conjugate vaccines. S. Enteritidis COPS-FliC conjugates were able to elicit protective antibodies in preclinical studies with both components of the conjugate elicit Salmonella-specific immunity. This work is being done in partnership with Bharat Biotech of India and the Wellcome Trust.

The Novartis Vaccines Institute for Global Health (NVGH) is developing a bivalent vaccine candidate for both S. Typhimurium and S. Enteritidis, using the GMMA platform for vaccine production. The GMMA methodology is feasible for making vaccines for a variety of Gram-negative bacteria, including Salmonella. Bacterial genetic modifications are introduced into the production strains to increase membrane blebs of small (50 to 90nm) immunogenic particles (GMMA) and to detoxify lipid A. In animal studies, GMMA are more immunogenic at comparable doses than glycoconjugate vaccines. Salmonella GMMA reactogenicity is untested in humans, although Phase 1 clinical trials of a Shigella sonnei GMMA are currently underway. The GMMA’s ease of manufacture and dose-sparing potential has influenced NVGH’s prioritization of a bivalent GMMA formulation over a bivalent O-antigen polysaccharide CRM197 conjugate vaccine approach. The NVGH bivalent conjugate vaccine is available for further development if the GMMA platform does not perform as expected.

The University of Birmingham in the United Kingdom has proposed a protein-based vaccine candidate consisting of outer membrane protein (Omp), specifically OmpD, purified from whole bacteria. Studies of porin-deficient bacteria identified that OmpD (absent from S. Typhi) is a viable target for antibody protection against iNTS. In 1992, the US National Institutes of Health (NIH) published research on an NTS conjugate vaccine for S. Typhimurium that linked O:4 to tetanus toxoid (O:4-TT). No further work has been published, nor have other plans been made publically available.

Microscience Limited has published results from the only live attenuated NTS vaccine candidate to be tested to date in humans. The vaccine, WT05, is derived from a gastroenteritis-associated strain of S.
Typhimurium and is attenuated by deletion of the *aroC* gene and the *ssaV* gene. A Phase 1 clinical trial found prolonged stool shedding in volunteers for up to 23 days, so the candidate has not been tested further. It should be noted that several other vaccines against *S. Typhimurium* and *S. Enteritidis* are available and/or under development for use in veterinary medicine and commercial food production, particularly in the raising of livestock and other key animal carriers, most notably chickens.

Table 1: Development Status of Current Vaccine Candidates (POC = Proof-of-concept trial)

<table>
<thead>
<tr>
<th>Candidate Name/Identifier</th>
<th>Preclinical</th>
<th>Phase I</th>
<th>Phase II</th>
<th>POC</th>
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References